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DEVELOPMENT OF NAILHEAD SPOT OF TOMATOES DURING TRANSIT AND MARKETING [1]

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INTRODUCTION

Ever since the passing of the food products inspection law of August 10, 1917, which authorized the Secretary of Agriculture to investigate and certify to shippers as to the condition of fruits and vegetables received on important markets, more and more attention has been focused upon the diseases of perishable products that cause damage during transit. As has been pointed out by Shear (*5*):[2]

> Few people, even pathologists, realize what enormous quantities of fruits and vegetables are lost through disease, decay, and other preventable causes, between the producer and the consumer. * * * It is considered a very serious matter to have growing crops injured or destroyed in the field, but how much more serious it is to have them destroyed after all the additional labor and expense of harvesting, packing and shipping.

The tomato is among the most perishable products on the market and is subject to such a wide variety of maladies that growers, dealers, and consumers alike suffer great financial loss each year. Inquiries and complaints received from interested persons brought to the attention of pathologists the necessity for a special consideration of the diseases of the tomato in transit. Consequently, Rosenbaum (*2*) conducted experiments and collected many valuable data relating to the origin and spread of tomato fruit rots in transit. After making a special investigation of the disease known as nailhead spot,[3] he (*3*) concluded that new infections in the field seldom occur on mature fruit or on fruit more than 6 inches in circumference, and that mature, green, healthy tomatoes do not develop new nailhead spots during transit. This was contradictory to the opinion held by many growers and by Rands (*1*), whose observations on this disease on the market indicated that it developed during transit.

The nailhead-spot disease is characterized by small, circular, slightly depressed, grayish brown to brown superficial spots produced on the fruit. In the early stages the spots are flat and tan in color (pl. 1, A and B), but as they enlarge the margins become more definite and dark brown to almost black and the centers change to gray or grayish brown and are definitely sunken (pl. 1, C and D).

[1] Received for publication Sept. 17, 1928; issued February, 1929. Contribution from the Office of Horticultural Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, and the Department of Botany, University of Chicago, cooperating. The field work reported in this paper is closely allied to the tomato nailhead-rust investigations carried on by the Bureau of Plant Industry in cooperation with the Florida Agricultural Experiment Station.

[2] Reference is made by number (italic) to "Literature cited," p. 145.

[3] Caused by *Macrosporium tomato* Cooke, according to Rosenbaum.

For many years the nailhead-spot disease has caused great loss of Florida tomatoes on the market as well as in the field. Since the inauguration of shipping-point inspection by the Bureau of Agricultural Economics, many discrepancies between the shipping-point and the receiving-market reports on the same car have shown a need for further investigation of this important disease. During the last three seasons the writers have studied the various phases of the development of nailhead spot as it occurs in the field in Florida, in transit, and on the northern markets. The work here presented was done primarily to determine whether new nailhead spots may develop on commercially packed fruit during transit, and to ascertain the rate of development of any such spots and also the rate of enlargement of various-sized nailhead spots present at shipping time.

There are few data available on the percentage of loss caused by nailhead spot alone, because many reports on loss of tomatoes caused by nailhead spot are given in conjunction with that caused by early blight. In 1918 Sherbakoff (*6*) stated:

> The early blight, sometimes called macrosporal spot, brown rust, or nailhead rust, spots the leaf, veins, and fruit, of the tomato plant. The leaves are eventually more or less seriously injured, sometimes killed, and the fruit * * * decayed. * * * On lowlands where the plants make a dense growth and heavy dews occur the disease may spot nearly every fruit in the field.

More recently Weber and Ramsey (*7*) conservatively estimated the average loss to be 10 to 15 per cent, and in some seasons much greater loss has occurred.

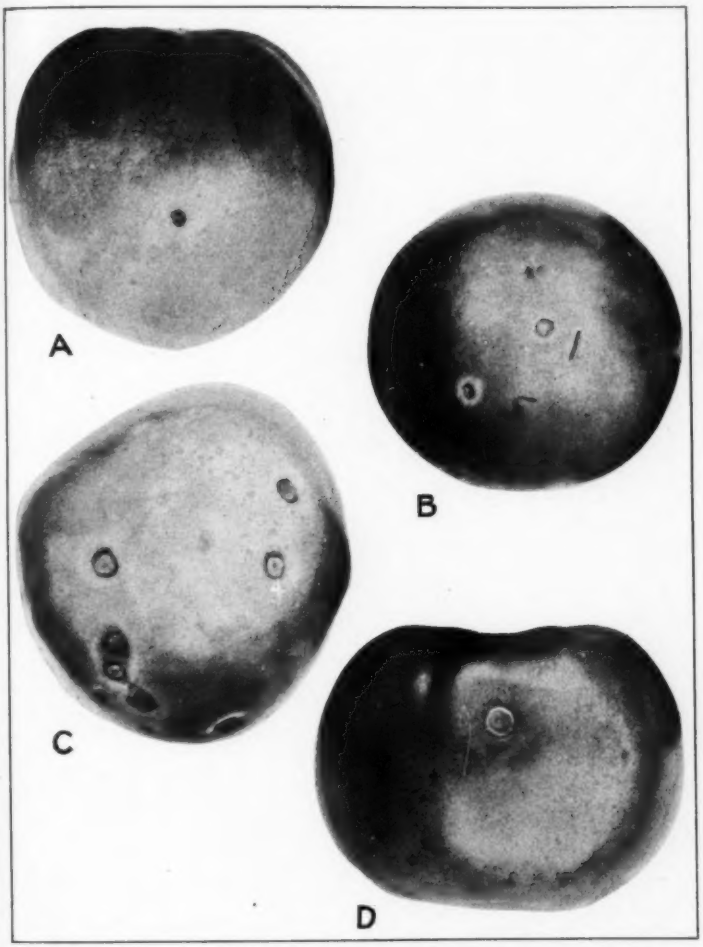
Inspections of carload lots of tomatoes on the receiving markets of the North show a wide variation in the percentage of nailhead spots present during different seasons. In some cars less than 1 per cent of the fruit was infected, while in others practically every fruit was spotted. Data [4] compiled from receiving-point inspection certificates of the Bureau of Agricultural Economics covering Florida tomatoes during the season of 1919 show that of the 116 cars in which nailhead spot was present 90 per cent of the fruits in 1 car had from 2 to 10 spots, 9 cars showed 30 to 50 per cent infection, and 106 cars showed 1 to 25 per cent infection. In 1920, 108 cars of Florida tomatoes showed an average of 13 per cent infection, and in 1921, 38 cars showed an average of 16 per cent infection.

The loss incurred on account of nailhead spot present in shipments of tomatoes is twofold: First, the market value of the stock is greatly reduced because of the appearance of the fruit; and secondly, the lesions produced by nailhead spot are subject to secondary invasion by decay-producing organisms. These organisms frequently cause a high percentage of loss while the tomatoes are in transit and while the green fruit is being held in the ripening rooms at the northern markets.

METHODS AND AIM

Mature green tomatoes were selected from the commercial stock in the packing houses in Florida for all test shipments. The fruits selected were examined individually for defects and disease of all

[4] COONS, G. H. DISEASES OF FIELD AND VEGETABLE CROPS IN THE UNITED STATES IN 1921. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Bul., Sup. 22: 322. 1922. [Mimeographed.]
HASKELL, R. J., and WOOD, J. [I.]. DISEASES OF FIELD AND VEGETABLE CROPS IN THE UNITED STATES IN 1919. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Bul., Sup. 10: 210. 1920. [Mimeographed.]
——— and WOOD, J. I. DISEASES OF FIELD AND VEGETABLE CROPS IN THE UNITED STATES IN 1920. U. S. Dept. Agr, Bur. Plant Indus. Plant Disease Bul., Sup. 16: 220. 1921. [Mimeographed.]



A.—2-mm. nailhead spot that developed during a transit period of 6 days. B.—Growth of small nailhead spots in storage. The 1-mm. spot measured 3 mm. at the end of 7 days in storage, whereas the 0.5-mm. spot increased to 2 by 3 mm. These spots showed the uniform color and irregular margin characteristic of actively growing lesions. C.—Typical nailhead spots of the intermediate stage, showing change of color, greater depression, and regular margin. D.—Typical 5-mm. nailhead spot that had ceased growing. The characteristic sunken gray center and dark-brown regular margin are shown.

kinds, and in order to avoid confusion and contamination only those that were practically free from diseases other than nailhead spot were used. The diameter of each spot was measured in millimeters and the size was marked in india ink at the side of the lesion. (Pl. 1, B.) The tomatoes thus examined and measured were wrapped and packed in 6-basket crates, according to commercial practice. These crates were then expressed to Chicago, Ill., where each spot was remeasured, especial care being taken to note the presence of any new spots that had not been visible at the time of shipping. In this manner a careful check has been made of representative samples of Florida tomatoes for the last three years. Crates of practically disease-free tomatoes and of those having as many as 1,107 nailhead spots have been studied. Usually fruits that showed only a few spots per specimen were selected, but occasionally a tomato with many spots was used for the sake of comparison. (Pl. 1, C.) One tomato on which 54 nailhead spots were present at the time of shipping, had on arrival at Chicago 107 additional spots. This, of course, is an extreme case, but it is of value in showing the range of possibilities.

On arrival at Chicago the tomatoes in the experimental shipments were divided into three lots, according to maturity. Although the maturity varied according to the season and the length of the transit period, 25 to 50 per cent of the stock was ripe on arrival, 15 to 25 per cent was turning, and 25 to 60 per cent was green. The green and turning stock was held at approximately 70° F. until ripe and the development of the nailhead spots during the storage period was noted. The stock just beginning to ripen on arrival was usually ripe after a storage period of 7 days, and the greenest stock ripened within 14 days. By numbering each spot and the individual fruits when they were received at the laboratory it was possible to observe the development of spots in some stock through a period of 14 days in the ripening room. These records give a complete history of the development of nailhead spots of tomatoes from the time the fruit was packed in Florida to the end of the transit and ripening periods.

In order to make sure that the rate of development of the nailhead spots was not influenced by the presence of secondary Macrosporiums or other organisms such as Phoma, it was necessary to make isolations from numerous lesions. During the progress of this work several hundred cultures were made from the various types of nailhead spot to check diagnoses. These types are not considered in this paper, however, which is confined to a study of the type of spot that constitutes a blemish merely, and is usually less than 6 mm. in diameter.

DEVELOPMENT OF NAILHEAD SPOT IN TRANSIT

During 1925, the first year of the study of the transit, market, and storage phases of nailhead spot, the test shipments were sent out from Miami, Fla. The average transit period necessary for the tomatoes to reach the Chicago laboratory was 5¼ days. The tomatoes used were all of commercial size and maturity, no fruit being less than 2 inches in diameter (more than 6 inches in circumference). In four representative crates of tomatoes there was a total of 1,211 spots, 422 of which were developed during the transit period. The

tomatoes on which these new spots were distributed represent 19.4 per cent of those shipped. Spots of all sizes present at the time of shipping enlarged to some extent, the rate of enlargement being inversely proportional to the original size of the spots. The new spots enlarged most, and those 3 mm. or more in diameter developed least. (Pl. 1, D.)

The relative growth of various-sized spots is shown graphically in Figure 1. From this figure it is apparent that the increase in diameter of the spots in the four crates is very similar. There are so many factors that might influence the rate of development of the spots in the individual crates that it is unwise to try to explain the variation in the rate of growth of the same-sized spots in the different crates. The differences due to varying length of the transit period are perhaps the most obvious and easily explained. For example, in crate 3, which was in transit only four days, there was, generally speaking, less growth than in any of the other three crates. However, crate 4, in which the greatest increases occurred, was in transit only five days, while in crates 1 and 2, which were in transit six

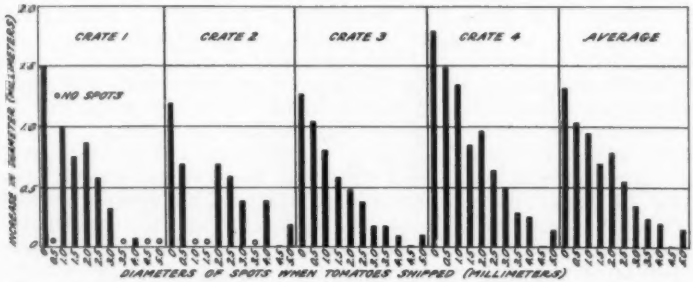


FIG. 1.—Increases in diameters of various-sized nailhead spots on tomatoes during the transit periods of crates shipped in 1925

days, there was no great development. From these facts it is apparent that the length of the transit period is not the only important factor in determining the increase in growth during transit. Variation in the different lots of tomatoes and environmental conditions not under control are also important factors in determining the rate of development of nailhead spots. Since this is true, the values shown in Figure 1 and the others to follow are not strictly comparable but give an accurate picture of the rate of growth of the spots under the field, transit, and marketing conditions to which Florida tomatoes are subjected during March, April, and May.

In four representative test crates of tomatoes shipped from Homestead, Fla., in 1926 there were 1,922 nailhead spots when the fruits were received on the Chicago market. Of this number, 211 were new spots that developed in transit on 17.7 per cent of the fruits shipped. The average diameter of the new spots was 1.58 mm. There were 1,711 spots measured and included in the crates at the shipping point. The relative increase in diameters of various-sized spots in the four crates is shown graphically in Figure 2. Compared with Figure 1, the average for 1926 is greater than that for 1925, although the bars are very similar.

Because of weather conditions unfavorable for the development of nailhead spot, and because much of the acreage on the east coast was planted to the Marglobe variety, which is highly resistant to nailhead spot, there was little material available for test shipments in 1927 until the latter half of the season. In three typical crates sent from Homestead, Fla., to the Chicago laboratory there were 810 nail-

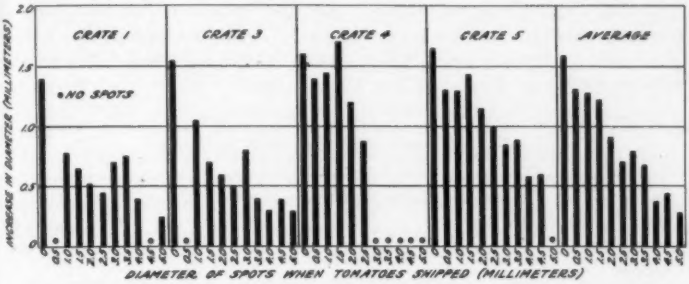


FIG. 2.—Increases in diameters of various-sized nailhead spots on tomatoes during the transit periods of crates shipped in 1926

head spots, 23 of which developed during transit on 4 per cent of the tomatoes shipped.

In Figure 3 the development of the spots in the three representative crates is shown. The spots on an average showed less increase in diameter during transit than those of either previous year. This may be accounted for largely by the unfavorable conditions mentioned above and the short transit period.

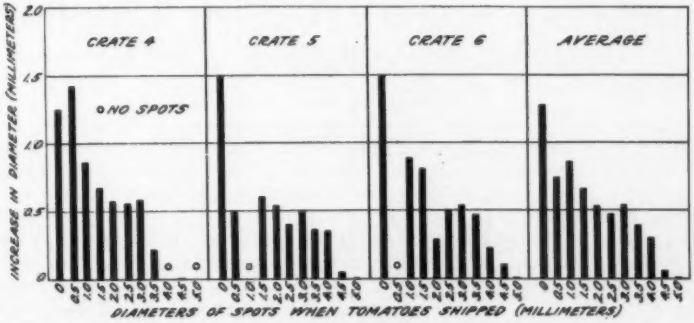


FIG. 3.—Increases in diameters of various-sized nailhead spots on tomatoes during the transit periods of crates shipped in 1927

As shown by the individual crates during the three years 1925 to 1927, inclusive, the increase in diameter of the spots usually varies with the length of the transit period. In Figure 4, which shows the average increases for the years mentioned, it will be noted that with the exception of a few of the larger spots less increase was made in 1927, when the average transit period was 3⅔ days, than in 1925, when it was 5¼ days. The greatest average increase was made during 1926, when the average transit period was 6¾ days. Table 1

shows the sizes of the various spots at the end of the transit period for each of the three years. It appears, therefore, that under approximately the same conditions the enlargement of nailhead spots of tomatoes during transit varies directly with the length of the transit period. Reduced to average increases in diameter per day during transit, the results for the three years are shown in Figure 5. The bars here presented are very similar in most respects and show clearly that the average increase per day varies inversely with the size of the spot at the time of shipping. The average daily increase in diameter of the nailhead spots during transit for the three years is shown in Table 2.

TABLE 1.—*Number and average diameters of nailhead spots on tomatoes at the end of the transit periods, 1925 to 1927*

Original diameter of spot	Number and average diameter of spots at end of transit period in—						3-year summary	
	1925		1926		1927			
	Number	Average diameter	Number	Average diameter	Number	Average diameter	Total number	Average diameter
Mm.		*Mm.*		*Mm.*		*Mm.*		*Mm.*
0	422	1.32	211	1.58	23	1.28	656	1.40
0.5	30	1.56	72	1.81	13	1.25	115	1.68
1.0	160	1.96	469	2.27	69	1.86	698	2.16
1.5	93	2.21	360	2.72	97	2.17	550	2.54
2.0	193	2.79	409	2.91	143	2.53	745	2.80
2.5	68	3.04	194	3.20	170	2.98	432	3.09
3.0	119	3.34	115	3.78	173	3.54	407	3.53
3.5	13	3.74	32	4.17	85	3.90	130	3.95
4.0	72	4.20	40	4.37	22	4.30	134	4.27
4.5	3	4.50	10	4.94	12	4.56	25	4.70
5.0	38	5.14	10	5.28	3	5.00	51	5.16

TABLE 2.—*Average daily increases in the diameters of nailhead spots on tomatoes during transit, 1925 to 1927*

Original diameter of spot	Average daily increase	Original diameter of spot	Average daily increase
Mm.	*Mm.*	*Mm.*	*Mm.*
0	0.25	3.0	0.11
0.5	.19	3.5	.10
1.0	.19	4.0	.05
1.5	.17	4.5	.03
2.0	.14	5.0	.02
2.5	.11		

It is of special interest to note that the average increase of the new spots developed during transit is at the rate of 0.25 mm. per day. Thus a perfectly smooth mature green tomato that has no visible spots at the time of shipping may develop nailhead spots 1 mm. in diameter by the end of four days in transit. Since the average transit period for most Florida tomatoes is about six days, the newly developed spots may attain a diameter of 1.5 mm. by the time they reach the northern markets. (Pl. 1, A.) Spots of this size are readily visible on the fruit, and for this reason an inspector's certificate issued at the receiving point may vary to a marked degree from the

one issued at the shipping point for the same stock. The percentage of new spots that develop during a given year varies to a great extent with the weather and probably somewhat with the varieties shipped. For example, in 1925, 35 per cent of the 1,211 spots studied were developed in transit, and in 1926, 11 per cent of the 1,922 spots studied were developed in transit, whereas in 1927, when weather unfavorable for the development of nailhead spot prevailed, only 2.8 per cent of the 810 spots studied were developed in transit.

The question whether it is possible to select and pack tomatoes that will show no spotting on arrival at the northern markets often arises in connection with the work of the Food Products Inspection Service. The following brief summary of an experiment indicates the probabilities:

Out of a lot of fancy Globe tomatoes that showed less than 1 per cent infection by nailhead spot in a packing house at Palmetto, Fla., 144 tomatoes were selected that were free from all blemishes and evidence of disease. These commercially perfect tomatoes were wrapped and packed in a 6-basket crate and shipped to Chicago. A careful exami-

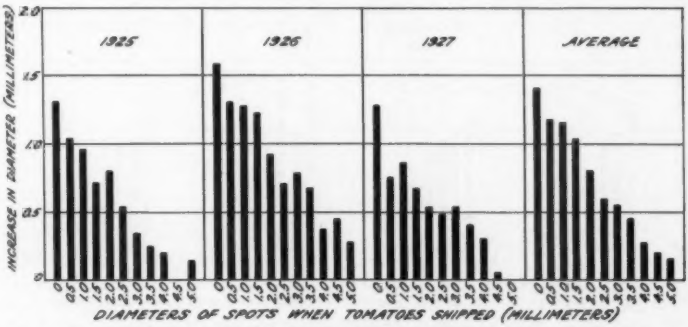


FIG. 4.—Yearly average increases in diameters of various-sized nailhead spots on tomatoes during transit, 1925–1927, and the average for the three years

nation on arrival showed that 6 nailhead spots averaging 1 mm. in diameter had developed during the 6-day transit period. The crate was repacked and held at a room temperature of approximately 70° F. for seven days. The tomatoes were again examined and it was found that 12 more nailhead spots had developed, making a total of 18 spots. The average diameter of the spots developed in storage was 1.6 mm., whereas that of the 6 spots developed in transit was 2 mm. at the end of the storage period. While these 18 spots were not in this instance on 18 different tomatoes, such a distribution might occur, in which case the most carefully selected tomatoes would show 4 per cent infection on arrival at market and 12.5 per cent infection after seven days' storage.

As a further example of what may happen to tomatoes during transit and during storage in the ripening rooms at the receiving market, the following experiment conducted in 1925 is worthy of consideration: One standard crate of 144 tomatoes and one of 180 tomatoes were carefully inspected, marked, and packed at Goulds, Fla., and shipped to New York. On their arrival in New York after six days in transit 5 new nailhead spots were found in the crate of

144's and 4 new ones in the crate of 180's. The two crates were then placed in a commercial ripening room and held for seven days. A careful inspection at the end of this time showed that a total of 69 new nailhead spots had developed in the crate of 144's since leaving Florida, and 171 spots developed in the crate of 180's. Most of the fruits had only 1 or 2 spots, but several had as many as 8 new ones.

DEVELOPMENT OF NAILHEAD SPOT IN STORAGE

After the tomatoes were examined upon arrival at Chicago and the nailhead spots remeasured, many of the fruits were rewrapped and placed in storage at room temperature. Observations and measurements were made from time to time to ascertain whether further development occurred in storage and, if so, what relation this bore to development in transit.

The average transit period for the crates thus stored during the three years was 5½ days. The average increase in growth during the 5½-day transit period was much greater in the case of all sizes of spots

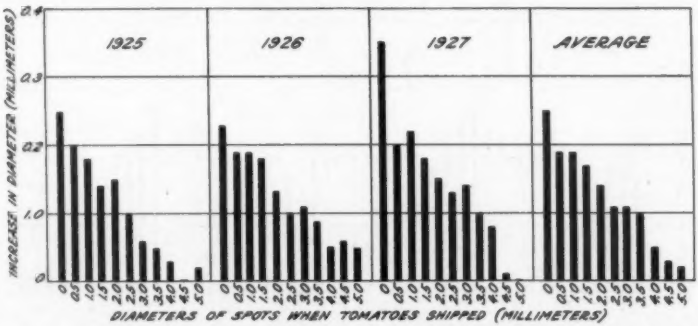


FIG. 5.—Average daily increases in diameters of various-sized nailhead spots on tomatoes during transit, 1925–1927, and the average for the three years

than was the corresponding increase during the 14-day storage period following transit. A comparison of the average increase during the two periods is shown in Table 3.

TABLE 3.—*Diameters of nailhead spots on tomatoes after 5½-day transit, 7-day storage, and 14-day storage periods*

Diameter of spots at shipping	Average increase in diameter during transit	Diameter of spots on arrival at destination	Average increase in diameter during 7-day storage period	Diameter of spots at beginning of second week of storage	Average increase in diameter during 14-day storage period	Diameter of spots at end of 14-day storage period
Mm.	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*
0	1.26	1.26	0.68	1.94	0.94	2.20
0.5	1.19	1.69	.36	2.05	.64	2.33
1.0	.92	1.92	.56	2.48	.57	2.49
1.5	.82	2.32	.40	2.72	.09	3.01
2.0	.68	2.68	.25	2.93	.45	3.13
2.5	.55	3.05	.16	3.21	.28	3.33
3.0	.50	3.50	.18	3.68	.26	3.76
3.5	.46	3.96	.16	4.12	.16	4.12
4.0	.26	4.26	.17	4.43	.17	4.43
4.5	.38	4.88	.13	5.01	.25	5.13
5.0	.23	5.23	.11	5.34	.21	5.44

The increase during the first week of storage was greater than that during the second week, though the difference was less than that between transit and storage. The smaller sized spots, such as 0 and 0.5 mm. grew more during the second week of storage than those that had diameters of 2.5 mm. or over when shipped. For example, the average increase of the 0-mm. spots for the first week was 0.68 mm. and for the second week 0.26 mm., whereas the average increase of the 3-mm. spots for the first week was 0.18 mm. and for the second week 0.08 mm. In most of the spots of 3.5 mm. or above there was little or no growth during the last week of storage.

The variation in rate of growth is shown in Figure 6, which illustrates the average daily rate of growth for the three years during the 5½-day average transit period, a 7-day storage period, and a 14-day storage period. It will be noted that the rate of growth during transit is much greater than that during either storage period, running from twice as great as that of the 7-day period in the case of the spots 4 mm. or larger to three times as great in the case of the new or 0-mm. spots. The rate of growth as well as the total average increase varies inversely with the size of the spots.

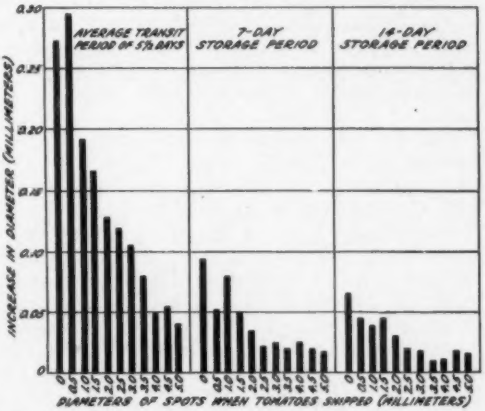


FIG. 6.—Average daily increases in diameters of various-sized nailhead spots during transit and storage periods for the years 1925, 1926, and 1927

The relation of maturity to the development of nailhead spot in transit and storage is discussed elsewhere in this paper. However, these data showing the decrease in amount and rate of growth with the increasing length of the storage period seem to uphold the observation that the rate of development of the nailhead spot decreases as the tomato ripens.

There seems to be some other factor, however, operating to limit the size of the spots, for even in green fruits the larger spots grow more slowly, and nailhead spots rarely reach a diameter of over 6 mm. without the presence of a secondary organism. Table 3 shows that this tendency of the growth rate to be correlated inversely with the size of the spot may also operate to reduce the rate of growth during the second week of storage, for it is evident that after the first week in storage the spots are all nearer their maximum size.

Observations of the rate of growth of the larger spots on green fruit would lead one to expect that the 3-mm. spot that had increased to 3.68 mm. by the end of the first week of storage and the 3.5-mm. spot that had increased to 4.12 mm. would now grow at a slower rate and show little additional growth after another week of storage. Moreover, one would expect that the growth of the 0-mm. and the

0.5-mm. spots, which are now practically 2-mm. spots, would be retarded. However, Table 3 shows that if the new size of any spot is taken on arrival and considered the size of the next larger sized spot (original size at shipping), in no case did it grow as much during the week of storage as did the original spot of the size during the 5½-day transit. For instance, the 0.5-mm. spots grew on an average 1.19 mm. in transit; that is, they were 1.69 mm. in diameter on arrival. Considering these as 2-mm. spots, we find that their subsequent increase of 0.36 mm. in 7-day storage is less than that of the 2-mm. spots, which grew 0.68 mm. in 5½-day transit. Yet if only the size of the spot was determining the rate and amount of growth, it would be expected that the figures showing increase in storage would not be consistently smaller than those showing the increase in transit of spots of the corresponding size.

These data, together with those shown in Figure 8, indicate that at least part of the cessation in growth is connected with the ripening of the fruit. The consistent decrease in amount and rate of growth with increase in the size of the spot shows, however, that there is some other factor tending to limit the size of nailhead spots. Rate of development varies inversely with the size of the spot and the maturity of the fruit.

During the seasons of 1926 and 1927 storage experiments were conducted in Florida also. There was available a ripening room that compared very favorably in respect to temperature and humidity with the ordinary commercial ripening rooms in the northern markets. Commercially mature green tomatoes selected from the packing house were wrapped and stored in this ripening room, and a careful check on the development of new spots and the amount of enlargement of the old ones was kept for comparison with similar stock shipped to Chicago. The results obtained from the various observations are best shown by three representative crates held in storage in 1927 as compared with three crates of the same stock shipped to Chicago. The average storage was for a period of seven days at a temperature ranging between 70° and 85° F. with an average relative humidity of 88 per cent.

The results obtained from this work show that as a rule the average increase in diameter of the spots was slightly greater in the stored than in the shipped stock. However, there are two important factors that would tend to cause this difference. In the first place, the stored stock was held approximately three days longer than the transit period for the shipped material, and secondly, the relative humidity in the ripening room, as well as the temperature, was uniformly higher than that existing in a car of tomatoes in transit. When the possible influence of these two factors is taken into consideration, the indications are that the rate of growth of the spots present in the two lots of tomatoes was very much alike.

The most interesting variation between the stored stock and that shipped was in the number of new spots that developed after the tomatoes were packed. Of the 810 spots present at the receiving market, 23, or almost 3 per cent, developed during transit; whereas of the 568 spots present in the storage stock, 65, or slightly over 11 per cent, developed after the tomatoes were placed in storage. Since high humidity and temperature favor the germination of Macrosporium spores on the fruit, it is probable that these factors are

largely responsible for the greater number of new spots that developed in storage.

The results of the storage work of 1926 were very similar to those reported for 1927. In a typical lot of mature green tomatoes taken directly from the packing house and stored in the ripening room until all of the fruits were ripe, 14 per cent of a total of 144 spots developed after the stock had been placed in storage. The average diameter of the new spots was 1.3 mm., as compared with 1.16 mm. in 1927. The mean temperature for the period was approximately 70° F. and the average relative humidity was 89 per cent. Several lots of tomatoes held under similar conditions at various times have given practically the same results, thus indicating that the figures cited are representative for the given conditions.

RELATION OF SIZE OF TOMATOES TO DEVELOPMENT OF NAILHEAD SPOT IN TRANSIT

One of the greatest obstacles to successful commercial handling of tomatoes is the fact that there is no clear-cut practical way of determining the maturity of tomatoes in the field. Although there are a few characteristics of approaching maturity that can be discerned by a trained man, these are of little use to the unskilled laborers who do practically all of the picking in Florida. Consequently, the chief criterion used for determining the proper stage of maturity is the size of the fruit. Under ordinary conditions there is, of course, a rather close correlation between size and maturity, but a wide range of variation is possible and actually occurs in commercial practice.

Rosenbaum and Sando (*4*) found that the age of the tomato fruit was a much better indication of maturity than size, and that maturity was the most reliable factor in determining infection by Macrosporium. The cuticle increases in thickness as the fruits approach maturity, and the work of Rosenbaum and Sando indicated that there was a definite correlation between maturity and resistance to skin puncture and infection. Resistance to infection was found by these investigators to be manifest when the fruits attained a circumference of 6 inches, at which time a pressure of 4 to 5 gm. was necessary to puncture the skin with a needle 78 μ in diameter. Thus, their experiments indicated that resistance to infection by the more mature fruits is due to a thickening of the cuticle which offers mechanical resistance to the infection hyphae. The field, transit, and laboratory work of Rosenbaum (*3*) indicated that few if any nailhead spots developed on tomatoes 6 inches or more in circumference.

Although it is true that most nailhead spots are found on immature stock, the present work will not permit a sharp line of demarcation to be drawn between the susceptible and nonsusceptible fruits on the basis of size. All of the tomatoes packed commercially in Florida are 6 inches or more in circumference. The smallest size packed is 216 per crate, and the average diameter of these tomatoes ranges from 2 to 2¼ inches, which is more than 6 inches in circumference. In the work here presented, covering three years of field, transit, and market observations and experiments, a high percentage of new infections has been found in many lots of tomatoes of various sizes, ranging from 2 to 3½ inches in diameter. If the stage of maturity is the primary factor in determining resistance to infection, then it is

probably variation in size of fruit of the same age that accounts for the development of spots in the larger sized fruits. A tomato 2½ inches in diameter may be less mature than one 2 inches; consequently, it is possible to find a larger fruit infected while a smaller one remains clean. Moreover, among fruits of the same size there may be enough difference in age to make some highly resistant to infection, while others are quite susceptible.

During 1927 an accurate record of the diameters of several hundred tomatoes was kept. Crates made up of a mixture of all sizes were shipped to Chicago to ascertain the rate of enlargement of the nailhead spots present at shipping time in relation to the size of fruit, and the percentage of new spots that developed in transit. According to commercial practice in Florida, the tomatoes were divided

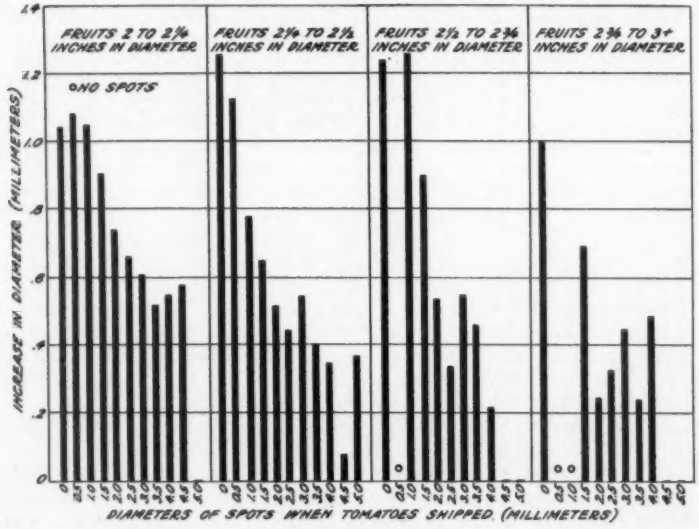

FRUITS 2 TO 2⅛ INCHES IN DIAMETER
FRUITS 2⅛ TO 2½ INCHES IN DIAMETER
FRUITS 2½ TO 2¾ INCHES IN DIAMETER
FRUITS 2¾ TO 3+ INCHES IN DIAMETER
NO SPOTS
INCREASE IN DIAMETER (MILLIMETERS)
DIAMETERS OF SPOTS WHEN TOMATOES SHIPPED (MILLIMETERS)

FIG. 7.—Average increases in diameters of various-sized nailhead spots on tomato fruits of different size during transit

into four lots according to size: Fruits 2 to 2¼ inches in diameter make a pack of 216 per crate; those 2¼ to 2½ inches, a crate of 180; 2½ to 2¾ inches, a crate of 144; and those 2¾ to 3 inches, a crate of 120. The results of this work, covering measurements of 2,359 nailhead spots, are shown in Figure 7. The most obvious result, as portrayed in this graph, is that the smallest size, represented by the 216's, shows a greater average increase in diameter of spots than any of the other sizes, and that the largest size, represented by the 120's, shows a smaller increase than any of the other sizes. The two intermediate sizes, 180's and 144's, show some variations, but in general these maintain a range fairly well between the results obtained from the two extremes.

The new spots that developed during the transit period are of special interest on account of their distribution among the different-sized tomatoes. In all there were 74 of these newly developed spots,

divided as follows: 33 per cent in the 216's, 39 per cent in the 180's, 27 per cent in the 144's, and 1 per cent in the 120's. It will be seen that the smaller sizes, viz, the 216's and 180's, contained 72 per cent of the newly developed spots and in general showed greater average increase in diameter of the old spots, while the larger sized 144's and 120's, the commercial fancy packs, had 28 per cent of the new spots. The figures arrived at in this work can not be considered absolute, on account of variations in seasonal and transit conditions, but they are indicative of what may be expected in an ordinary season. The results here reported confirm those of Rosenbaum in showing that the smaller tomatoes are more susceptible to infection by nailhead spot, but they conflict with his finding (*3, p. 13*) that "mature fruit picked green, as is usually done, and healthy at the time of packing, showed no new spots on their arrival."

All of the work done during the last three years has been with commercially mature green tomatoes, and in each season numerous

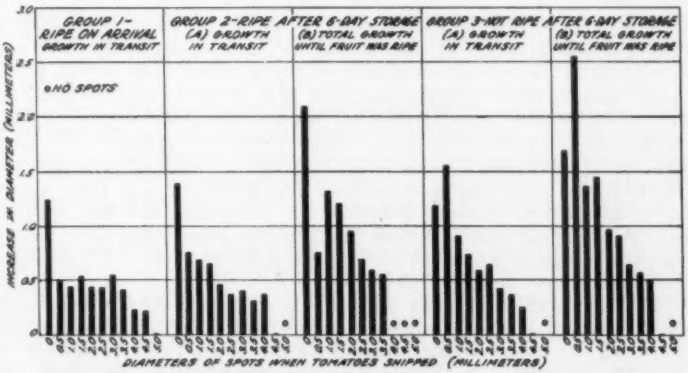

GROUP 1—RIPE ON ARRIVAL GROWTH IN TRANSIT
GROUP 2—RIPE AFTER 6-DAY STORAGE (A) GROWTH IN TRANSIT
(B) TOTAL GROWTH UNTIL FRUIT WAS RIPE
GROUP 3—NOT RIPE AFTER 6-DAY STORAGE (A) GROWTH IN TRANSIT
(B) TOTAL GROWTH UNTIL FRUIT WAS RIPE
NO SPOTS
INCREASE IN DIAMETER (MILLIMETERS)
DIAMETERS OF SPOTS WHEN TOMATOES SHIPPED (MILLIMETERS)

FIG. 8.—Average increases in diameters of various-sized nailhead spots during the transit and ripening periods of tomatoes, grouped according to degrees of maturity

nailhead spots that were not visible to the naked eye at the packing houses in Florida have been found in the stock on arrival at market. The greater number of spots occurred in the small sizes, but the size limit for new infections, placed at 6 inches in circumference by Rosenbaum, is manifestly a mistake, for were this correct no spots would be developed in commercially packed tomatoes during transit.

RELATION OF MATURITY TO DEVELOPMENT OF NAILHEAD SPOT IN TRANSIT

Data bearing upon the relationship of maturity to the development of nailhead spot during transit were obtained by numbering all fruits and keeping a separate record for each until it was ripe. The data thus obtained were used in separating the tomatoes into three groups according to their maturity as judged by the ripening period. The tomatoes that were ripe on arrival comprised the first group, those that ripened within six days after arrival the second group, and those not ripe after six days the third group. The average increase in diameter of all spots on tomatoes in transit in each group is shown in Figure 8. This figure shows that the average increase in diameter

of the spots in Group 1 was for the most part decidedly smaller than in the less mature stock in Groups 2 and 3. Especially is this true of the smaller sized spots ranging up to 2.5 mm. in diameter, which always represent the stage of most rapid growth of nailhead spots. Since in all instances there is a marked diminution in the growth rate of nailhead spots when they reach a diameter of approximately 2.5 mm., the spots ranging from 0 to 2.5 mm. are a much better criterion upon which to base growth rate than the spots 2.5 to 5 mm. On this basis it will be seen that the spots on the moderately green fruit represented by Group 2 showed an appreciable amount of development over Group 1, but considerably less than in the very green stock represented by Group 3. The actual difference in the amount of growth made in transit by the three different groups may be expressed in the sum total of the average increases in diameter of all spots in each group. Expressed thus, the spots in Group 1 made a total increase of 4.99 mm.; Group 2, 5.53 mm.; and Group 3, 6.78 mm.

Previous observations indicated that nailhead spots enlarge more rapidly in immature than in mature stock, but up to this time no accurate data have been available to prove this. The development of new nailhead spots during transit is also quite marked in the more immature stock as compared with the mature. In Group 1 there were 302 spots, only 1 of which (0.3 per cent) originated in transit; in Group 2 there were 248 spots, 11 of which (4.4 per cent) originated in transit; and in Group 3 there were 254 spots, 11 of which (4.3 per cent) originated in transit. These data, therefore, indicate that the stage of maturity has a direct bearing not only upon the rate of development of spots present at shipping time but also upon the number of new spots that develop during transit.

The total growth made up to the time when all tomatoes were ripe is also shown in Figure 8. The graph for Group 1 remains the same because all of the tomatoes ripened during the transit period of 3⅔ days. The tomatoes in Group 2 were not ripe, however, until after a storage of 6 days in addition to the transit period. The total growth of spots in the group is shown as Group 2a. Likewise, Group 3a, as shown in the graph, represents the total growth of the spots in Group 3 up to the time when all of the tomatoes were ripe, the ripening period for this group of green stock being 12 days in addition to the transit period of 3⅔ days. Here again the greatest growth is found in the greenest stock, Group 3a, but in this instance the tomatoes had an extra storage of 6 days over those in Group 2a, and this would account for part of the increased growth.

SUMMARY AND CONCLUSIONS

The present paper on nailhead spot represents the results of field, transit, and storage studies conducted from 1925 to 1927 with test shipments of tomatoes sent from Florida to Chicago, Ill.

Nailhead spot is one of the serious diseases of Florida tomatoes. It causes great loss of fruits in the field and packing houses, as well as during transit and marketing.

Nailhead spot is not limited to tomatoes 6 inches or less in circumference, as indicated by the work of Rosenbaum, but may develop on

fruits of any size, although most of the infections take place on the smaller tomatoes. Infection is correlated with maturity, and size is not necessarily indicative of maturity. The rate of development of nailhead spot is noticeably more rapid in green tomatoes, decreasing uniformly as the fruit matures. The number of spots occurring and the percentage of infected fruits is also greater in the greener tomatoes.

New nailhead spots may originate in transit or in storage on tomatoes of any size used in commercial pack in Florida. However, most new spots will be found in those composing the 216 pack, whose average diameter ranges from 2 to 2¼ inches, and in those of the 180 pack, whose average diameter ranges from 2¼ to 2½ inches. In 1927 these two packs contained 72 per cent of the newly developed spots, while the 144 and 120 packs contained only 28 per cent.

The percentage of fruits that developed new spots in transit varied from 4 in 1927 to 19.4 in 1925. The maturity of tomatoes shipped and the variation in seasonal conditions are important factors in determining the percentage of infection and the resultant economic loss occurring in any one year. The average diameter of the new spots that developed in transit during the three years under consideration was 1.4 mm. These spots are flat, somewhat irregular in outline, tan in color, and only skin deep.

The new nailhead spots enlarge more rapidly in transit and storage than those present when the tomatoes are shipped. During transit the rate of enlargement of the spots present at the time of shipping varies inversely with the size of the spot, the average daily increase in diameter ranging from 0.02 mm. in the 5-mm. spots to 0.25 mm. in the new spots. This fact shows that some factor other than maturity operates to limit the size of the nailhead spot. Storage experiments show that there is a decrease in rate of development as the size of the spot increases. The increase during the first week of storage was greater than that during the second week, but was much less than that during the average transit period.

The average nailhead spot has practically ceased to enlarge by the time it reaches a diameter of 3 mm., and only rarely does it attain a diameter greater than 6 mm. Of all the spots measured during the three years, less than 10 per cent were larger than 3 mm. in diameter.

The results of the experiments here recorded show clearly that there may be an appreciable increase in the number of spots occurring and in the percentage of fruits infected by nailhead spot during transit and storage. Even crates of carefully selected tomatoes entirely free from spots when shipped may show some spots on arrival at market.

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GREENHOUSE STUDIES ON THE RELATION OF AGE OF WHEAT PLANTS TO INFECTION BY PUCCINIA TRITICINA[1]

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INTRODUCTION

The resistance of wheat to leaf rust (*Puccinia triticina* Erikss.) and stem rust (*P. graminis tritici* Erikss. and Henn.) has been considered by most investigators to be a relatively stable character. Some, however, have noted that resistance to these rusts apparently may be modified by environmental conditions. In general, however, it has proved to be a definite, heritable character, the expression of which is no more affected by external conditions than that of many other characters. The inheritance of resistance either to leaf rust or to stem rust of wheat has received much study. The closeness with which the observed ratios in most of these studies agree with the calculated ones is proof that, with certain varieties of wheat and under certain conditions, the characters of resistance and susceptibility are very stable.

In spite of these facts, various investigators have noted that the reaction of certain varieties and hybrids of wheat or closely related grasses to rust sometimes undergoes a change, and the reaction of mature plants is different from that of seedlings. Stakman and Piemeisel (*7*)[2] found that seedling plants of certain grasses became infected with rust much more readily than old plants. Harrington and Aamodt (*1*) stated that a variety of wheat, the seedlings of which give a type 3 reaction to stem rust in the greenhouse, is resistant in the field. Hursh (*2*) stated that it has "been observed that certain varieties of wheat, such as Kota and Acme, are susceptible to a considerable number of biologic forms in the greenhouse, but that they seem to be fairly resistant in the field." His experiments led him to believe that one of the principal reasons for that reaction was the greater amount of sclerenchyma in mature plants than in seedlings. This acted as a mechanical barrier to the growth of the rust mycelium.

Melchers and Parker (*5*), in discussing the resistance of winter-wheat varieties to stem rust, noted that a strain of Crimean Kansas No. 2390, partially resistant in the rust nursery, gave no evidence of resistance in the greenhouse. They concluded that "plants showing certain effects when inoculated in the seedling stage may respond very differently to the same rust when they are subjected to it in the heading stage under field conditions." The same phenomenon

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[2] Reference is made by number (italic) to "Literature cited," p. 157.

was noted by Mains and Jackson (*3*) in their studies on physiologic forms of *Puccinia triticina*. They found many varieties of wheat to be only moderately resistant or even susceptible in the greenhouse, while they exhibited high resistance in the field.

Mains, Leighty, and Johnston (*4*) also reported that certain varieties of wheat appeared much more resistant to leaf rust in the field than in the greenhouse and stated that environmental conditions and vigor of the host undoubtedly are partly responsible for the difference. The same authors, in reporting on the inheritance of resistance to leaf rust in crosses between Kanred and Fulcaster winter wheats, noted a marked change in the reaction of F_2 plants to physiologic form 9 (p. f. 9). In the seedling stage a ratio of approximately one resistant plant to three susceptible ones appeared. At heading time many of those classed as susceptible in the earlier stage of growth were highly resistant. Such hybrids exhibited marked flecking accompanied by only a few small uredinia on the lower leaves. The F_3 data showed that the heterozygous F_2 plants changed in their reaction to rust, while the homozygous resistant and homozygous susceptible plants were constant in their respective reactions at all stages of growth.

The studies reported in this paper were started primarily to ascertain whether there were any varieties of wheat that changed in their reaction to leaf rust in a manner similar to that noted above for the Kanred-Fulcaster hybrids. Casual observation led the writers to believe that such changes occurred in certain varieties, for it was noted on numerous occasions that such varieties as Marquis, Nittany (Penn. 44), Trumbull, Shepherd, and Mammoth Red exhibited considerable resistance to *Puccinia triticina* p. f. 9 at heading time in the field, while seedlings of the same varieties proved very susceptible to the same form of rust in the greenhouse. Experiments, therefore, were outlined to ascertain the reaction of a number of varieties of wheat to leaf rust at different stages in the growth of the plant.

MATERIALS AND METHODS

These experiments were started in the fall of 1925, the first crop being grown during the winter of 1925–26 and the second during that of 1926–27. All plants were grown to maturity in the greenhouse, and all subsequent discussion refers only to the greenhouse results. Four-inch flower pots were used, previous experiments having shown that wheat plants grown in pots of that size are normal in every respect. A single plant was grown in each pot, thus allowing observations on individual plants at any time during their life.

The varieties used in this experiment were selected on the basis of their known reactions to *Puccinia triticina* p. f. 9 in the greenhouse, or because their field reactions to rust had indicated that they probably had changing reactions. Thus Kanred was selected because it was found to be highly susceptible to *P. triticina* p. f. 9 in the greenhouse while it often exhibited considerable resistance in the field. Harvest Queen was selected because it usually exhibits a high susceptibility to leaf rust both in the greenhouse and in the field. Fulcaster (susceptible strain), Blackhull, Michigan Wonder, and Hussar were selected because of the known variability of their reactions to leaf

rust. Fulcaster (resistant strain) and Kawvale were selected because they were known to be highly resistant to physiologic form 9 in all stages of growth. Mammoth Red, Trumbull, Shepherd, Nittany, Marquis, Hungarian, Bacska, Sibley, Banat, Michikof, Russian, Goldcoin, Iobred, Rupert, and Red Clawson were chosen because they often exhibit considerable resistance to leaf rust in the field, while in the seedling stage they had proved highly susceptible to form 9 in the greenhouse. Padui, Malakof, and Brown Bearded were added to the experiment because of the high susceptibility they usually exhibit in the field in Kansas. P721 usually has shown a leaf-rust reaction much like that of Kanred. It therefore was added to these experiments to ascertain whether the resemblance existed for plants of different ages. The first 13 of the foregoing varieties were studied during two separate seasons, while the last 14 were studied only in 1926–27.

Whenever possible, pure-line seed was used in making the sowings, but when this could not be secured the best seed obtainable was used. The plants of the 1926–27 experiments were grown from seed produced by the plants in the experiments of 1925–26, except in the case of the 14 varieties first used in 1926–27. Seed of those varieties was secured from the 1926 leaf-rust nursery and in most cases was not pure line, although of good quality and as pure as could be obtained by ordinary nursery procedure.

Ten plants of each variety, except Marquis and Nittany, were used in the experiments conducted in 1925–26. Twenty plants each of Marquis and Nittany were grown. In the experiments of 1926–27, 10 plants were grown of each variety except Marquis, Nittany, and Trumbull, of which 20 plants each were studied.

Physiologic form 9 of *Puccinia triticina* was used for the experiments of both seasons. This form was selected because of its wide prevalence in the southern Great Plains area and because it was the form to which certain varieties had proved resistant in the field and susceptible in the greenhouse. The rust culture used was derived originally from a single well-isolated uredinium and always gave the typical reaction for physiologic form 9 when tested on differential varieties. It was tested for purity at short intervals during both seasons. The stock culture was grown on seedling plants in small flowerpots and was transferred frequently enough to provide an abundant supply of fresh uredinial material at all times.

When the experimental plants had reached the desired stage of growth they were placed in a large moist chamber and the leaves were thoroughly moistened with a fine spray of tap water. A number of pots of heavily rusted seedlings then were shaken vigorously above the plants, so that a shower of spores fell on the leaves. The plants were left in the moist chamber for 48 hours and then placed on the greenhouse bench. Excellent infection usually resulted from the use of this technic. By this method a large number of plants can be inoculated simultaneously and a uniform infection obtained on virtually all leaves exposed to the inoculum.

The plants used in these experiments were inoculated during three stages of growth, here designated as the "seedling," "shooting," and "heading" stages. The seedling inoculation was made about one month after sowing, when the plant had five to seven leaves

and while the prophyllum or primary leaf was still green. The shooting-stage inoculation was made after the plants had passed the stage of very slow growth, which, in the greenhouse, is comparable to the winter period of dormancy out of doors. At this time growth was rapid and the plants had just begun to shoot. The third inoculation was made when the head emerged from the boot, or approximately one month before ripening.

Rust notes were taken on all plants within 14 to 16 days after inoculation. For each stage of growth a note was taken on the rust reaction of the uppermost and lowermost leaves of the same plant. In most cases it was necessary to use a range to designate the type of reaction. The average range of reaction for all plants of each variety at each stage of growth was then determined. (Tables 1 and 2.) The method of placing plants in five rust-reaction groups numbered from 0 to 4 was used. The principles laid down by Mains and Jackson (*3*) for the determination of the reaction of wheat plants to leaf rust were followed. Rust-free plants were given a classification of 0, completely susceptible ones were placed in class 4, and those of lesser degrees of susceptibility were placed in the intervening classes.

TABLE 1.—*Reaction of varieties of wheat to leaf rust (Puccinia triticina physiologic form 9) in different stages of growth and in different parts of the plant, in the greenhouse, Manhattan, Kans., 1926 and 1927*

Variety	Number *	Leaves	Classes of rust reaction at—			
			Seedling stage		Heading stage	
			1926	1927	1926	1927
Kanred	55-6-1	Lower	4	4	3	2-3
		Upper	4	4	3	2-3
Harvest Queen	5-2-2-2	Lower	4	4	4	3
		Upper	4	4	3	2-4
Fulcaster (susceptible)	47-2-34-7	Lower	4	4	1-2	1-2
		Upper	4	4	1-2	0-2
Fulcaster (resistant)	7-2-1-1	Lower	1-2	0-1	0	0-1
		Upper	0	0	0	0-1
Blackhull	Ks. 343	Lower	2-4	3	2-4	3
		Upper	2-4	2-3	3-4	3
Michigan Wonder	Ks. 2534	Lower	2-3	4	3-4	2-3
		Upper	2-3	2-4	3-4	3-4
Hussar	C. I. 4843	Lower	2-3	2-3	2-3	2
		Upper	2-3	2-3	2-3	2-3
Mammoth Red	Ks. 2517	Lower	4	4	0-3	2-3
		Upper	4	4	0-3	0-3
Trumbull	Ks. 2547	Lower	4	4	2-3	2-3
		Upper	4	4	2-3	0-2
Shepherd	Ks. 435	Lower	4	4	2	2-3
		Upper	4	4	2	0-3
Nittany	Penn. 44-3	Lower	4	4	0-3	0-2
		Upper	4	4	1-2	0-2
Marquis	C. I. 3641	Lower	4	4	0-2	2-3
		Upper	4	4	0-2	1-2
Kawvale	62-1-1	Lower	0-2	0-1	0-1	0-1
		Upper	0	0	0	0

* Numbers preceded by C. I., Ks., and Penn. are accession numbers of the Office of Cereal Crops and Diseases, the Kansas Agricultural Experiment Station, and the Pennsylvania Agricultural Experiment Station, respectively. Numbers not preceded by one of these are pedigree numbers designating single-plant selections grown in the greenhouse the preceding year.

TABLE 2.—*Effect of age and position of leaves on plant on the reaction of certain varieties of wheat to leaf rust (Puccinia triticina physiologic form 9) in the greenhouse, Manhattan, Kans., 1926–27*

Variety	Number [a]	Leaves	Classes of rust reaction of—	
			Seedling stage	Heading stage
Hungarian	C. I. 4830–2	Lower	4	2–3
		Upper	4	0–2
Bacska	C. I. 6156–3	Lower	4	3
		Upper	4	2–3
Sibley	C. I. 5066–1	Lower	4	2–3
		Upper	4	3–4
Banat	C. I. 4879–4	Lower	4	3
		Upper	4	3–4
Michikof	Selection 1	Lower	4	2–3
		Upper	4	1–2
Russian	Ks. 2587	Lower	4	2–3
		Upper	4	0–2
Goldcoin	Ks. 2567	Lower	4	2–3
		Upper	4	0–2
Iobred	Ks. 431	Lower	4	2–4
		Upper	4	0–3
Rupert	Ks. 2571	Lower	4	2–3
		Upper	4	0–1
Red Clawson	Ks. 2577	Lower	4	2
		Upper	4	0–1
Padui	Ks. 2467	Lower	4	3
		Upper	4	3–4
Malakof	C. I. 4898–4	Lower	4	3
		Upper	4	3–4
Brown Bearded	C. I. 3118–2	Lower	4	2–3
		Upper	4	2–4
P721–1		Lower	4	2–3
		Upper	4	3

[a] Numbers preceded by C. I. and Ks. are accession numbers of the Office of Cereal Crops and Diseases and the Kansas Agricultural Experiment Station, respectively.

EXPERIMENTAL RESULTS

The results obtained in the two years' experiments are summarized in Tables 1 and 2. Table 1 gives the reaction of the varieties used in both years. Table 2 gives the results for a number of varieties used only in 1926–27. It will be noted that in both tables the results for the seedling and heading stages are given, while the results for the shooting stage are omitted. This was done to give greater emphasis to the differences between the reaction of seedlings and mature plants. The reaction of plants in the shooting stage was variable, depending upon the variety. In some varieties it was the same as in the seedling stage, in others it resembled that of the heading stage, and in a few instances it appeared to be intermediate between those of seedlings and mature plants. Further studies on changes in reaction to rust are being conducted to ascertain just when they occur and how rapidly.

Table 1 shows definitely that marked changes in reaction to leaf rust occurred in some varieties in both years. Fulcaster 47–2–34–7, Mammoth Red, Trumbull, Shepherd, Nittany, and Marquis were markedly more resistant in the heading than in the seedling stage. While the average rust reaction of each of these varieties is not exactly the same, a remarkable increase in resistance in the older plants is clearly demonstrated. All these varieties were completely susceptible as seedlings, there being no necrosis or other signs of

resistance. In the heading stage they all showed marked reduction in the size and number of uredinia and a distinct yellow necrosis surrounding the uredinia. In the case of Marquis and Nittany the resistance in the heading stage was so marked that many leaves showed only numerous small yellow killed areas or flecks. (Pl. 1, A, D.)

In some varieties, such as Mammoth Red, Trumbull, and Shepherd, the reduction in the number of the uredinia was not so marked, but there was a decided reduction in size and an increased necrosis. It will be noted that the rust reactions of the lowermost leaves of Mammoth Red were classified 0 to 3 in 1926 and 2 to 3 in 1927. In both cases the necrosis was marked, but in 1927 small uredinia were present, while in 1926 only flecks occured. Although this seems to be a rather large variation, it is one principally of degree, for casual observation would have classed the seedling plants as susceptible and the mature plants as resistant.

Kawvale and Fulcaster 7–2–1–1 (pl. 2, B), the two highly resistant varieties included in this experiment, did not change appreciably in their rust reaction. They remained highly resistant in all stages of growth. The only notable difference in reaction was the slightly greater resistance exhibited by the upper leaves. On the other hand, Kanred and Harvest Queen, usually considered susceptible to physiologic form 9, did show a somewhat more pronounced resistance in the heading stage. This was evidenced not so much by a reduction in the number or size of the uredinia as by an increase in the necrotic areas surrounding them. In some plants this discoloration of tissues surrounding the uredinia was light green, giving them a rust classification of 3. In a few cases the necrosis approached the typical yellow type commonly seen in rust-reaction class 2. The reaction of Kanred, therefore, was somewhat like its field behavior, but the resistance shown by that variety in these experiments was not nearly so marked as that which it sometimes exhibits in the field. The resistance of Kanred in the field, however, may be due sometimes to the presence of some physiologic form of leaf rust to which it is resistant, since greenhouse experiments have proved that the variety is moderately resistant to some forms of leaf rust. On the other hand, Kanred often shows considerable resistance in the field when physiologic form 9 is the principal invader. It has been observed repeatedly in the rust nursery at Manhattan, Kans., that Kanred was heavily attacked by physiologic form 9 in the fall when the plants were small, while at heading time, in the spring, the same rows exhibited considerable resistance to the same form of rust. The resistance of the mature plants often is similar to the X type of infection noted by Stakman and Levine (*6*), there often being both large and small uredinia and necrotic and nonnecrotic areas on the same leaf. Isolations from the small uredinia usually are the same physiologic form as those from large uredinia.

Blackhull and Hussar in the seedling stage exhibit a moderate resistance of type 2 to physiologic form 9. It was thought that perhaps such varieties might prove highly resistant in the heading stage. They were used in these experiments to ascertain whether or not this was the case. The results, though not entirely uniform, demonstrate rather definitely that partially resistant varieties do not necessarily become more resistant as they grow older. While these varieties

showed some variation in reaction to rust at different stages of growth, there was no marked increase in resistance during the heading stage.

In the fall of 1926 the number of varieties was increased by 21, the rust behavior of each of which, in the field, indicated a degree of resistance somewhat like that shown by Marquis, Nittany, and Trumbull. Pure-line seed of all of these additional varieties was not available, and as a result it was necessary to discard seven from the experiment because of variability in their reaction to rust. Some of them contained both resistant and susceptible strains, which reacted consistently at all stages of growth. Others were susceptible in the seedling stage, but in the heading stage some plants were resistant, while others of the same variety were susceptible. Morphologically, the plants usually seemed to be all alike, but physiologically and pathologically they varied widely. Those varieties accordingly were eliminated from the experiments.

The reactions of the remaining 14 varieties are given in Table 2. It will be noted that seven proved to be much more resistant in the heading than in the seedling stage, while seven others showed less marked changes. The two years' work demonstrates beyond doubt that some varieties of wheat change in their reaction to certain forms of leaf rust from complete susceptibility in the early stages to a high degree of resistance in the heading stage. This partially explains some of the discrepancies that have been noted between the reactions of seedling plants in the greenhouse and nearly mature plants in the field to the same physiologic form of leaf rust. There is a very practical application of the results of these studies in that they demonstrate that investigators should not too rigidly confine their rust-resistance studies to seedling plants. The increasing resistance of certain hybrids and varieties raises the question whether testing such lines in the heading stage of growth would not lead to the saving of certain promising ones that might have been discarded because of their susceptibility to rust in the seedling stage. Investigators would be justified in saving certain agronomically promising varieties and hybrids, even though they were known to be rust susceptible in certain stages of growth, if they were resistant during the critical fruiting period.

REACTIONS OF LEAVES IN DIFFERENT PARTS OF THE SAME PLANT

These studies not only proved that plants of certain varieties of wheat reacted differently to leaf rust in different stages of growth, but also in numerous instances they revealed a marked difference in the reaction of leaves in different parts of the same plant. In both Table 1 and Table 2 the rust reactions of the lower and upper leaves are given for each stage of growth. An examination of the rust classifications given to the leaves in different parts of the plant shows that in most cases where differences exist the uppermost leaves proved to be more resistant than the lower ones. This difference in reaction to rust usually is not noticeable in very young plants, the reaction of the first few leaves following the prophyllum being the same as that of the first seedling leaf except in the case of resistant varieties. In the latter it usually happens that if any leaf of the plant shows any uredinia it is the prophyllum. This point is well illustrated by the data given in Table 1 for the seedling stage of the resistant selection

of Fulcaster and Kawvale, both of which are highly resistant to physiologic form 9. The reaction of the lower leaves of resistant plants in the heading stage is very similar to that of the prophyllum in the seedling stage, i. e., if any uredinia are found upon such plants they almost invariably occur on the lowermost leaves.

The same statement also applies in a general way to those varieties that change from complete susceptibility in the seedling stage to a marked resistance at heading time. This is shown in Table 1 by the heading-stage reaction of such varieties as Mammoth Red, Trumbull, Shepherd, and Marquis, and in Table 2 by Hungarian, Michikof, Russian, Goldcoin, Iobred, Rupert, and Red Clawson. It should be stated that where several plants of each variety are grown, as in these experiments, this reaction is difficult to show in a table. The data must be given in the terms of rust classes, and it is not possible to average these as definitely as desired. In lieu of this the range of reactions noted for the upper and lower leaves in both seedling and heading stages of growth is given. This often conceals in the data the remarkable behavior it is desired to show. For instance, the data do not show such distinct differences as those shown in Plate 1. Where such ranges of reaction as 0–3, 0–2, and 1–2 had to be assigned to certain varieties because of slight variations in 1 or 2 plants of the 10 or more of each variety studied, a casual observation of the upper leaves would seem to reveal a higher resistance than that shown by the data.

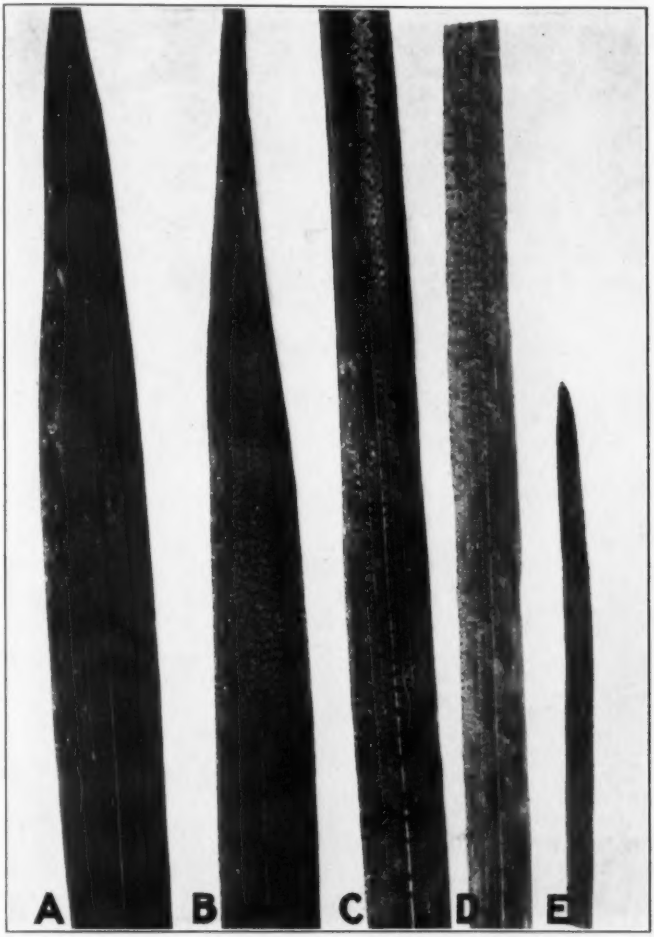
The general point that in varieties that become more resistant as they near maturity the uppermost leaves are more resistant than the lower ones is indicated in Tables 1 and 2. This reaction is sometimes so marked that the terminal or flag leaf often exhibits only a number of flecks or small killed areas, indicating infection, whereas the lower leaves carry considerable rust, although they also may have some necrotic areas around some small or minute uredinia. If only the upper leaves of such plants were considered the plants would be classed as highly resistant. Where this condition exists there is usually a gradual decrease in resistance from the top of the plant downward. There is, however, considerable variation in this, some plants showing rust on only two or three of the lowermost leaves, while others show considerable rust on leaves half way up the culms. This difference is sometimes specific for the variety, the plants of one variety having only a few rusted leaves near the base, while another variety has rusted leaves half way up the stem.

While the condition just discussed usually is observed in varieties that are more resistant in the heading than in the seedling stage, it is not seen in certain susceptible or partially susceptible varieties, such as Michigan Wonder, Blackhull, Hussar, Bacska, Sibley, Banat, Malakof, Brown Bearded, Padui, and P721. In such varieties it often happens that the uppermost leaves in the heading stage are more susceptible than the lower leaves. They have more and larger uredinia and there is less evidence of necrosis surrounding the uredinia in them than in the lower leaves. (Pl. 2, D.)

Besides the two types of rust reaction just discussed, another reaction that should be mentioned was observed. A typical example of this is shown in Plate 3. In this case the uppermost leaf shows a resistant reaction, the next two leaves below seem susceptible, and

Leaves of wheat varieties, showing greater resistance to leaf rust (*Puccinia triticina*, physiologic form 9) of mature plants and of upper leaves of the same plant. A.—Marquis. B.—Red Clawson. C.—Trumbull. D.—Nittany (Penn. 44). Throughout the plate: *a*, Flag leaf of mature plant; *b*, lower leaf of the same plant; *c*, prophyllum of seedling plant. Note the small white flecks on the flag leaf, showing that rust infection occurred but that very few uredinia developed

Leaves of wheat varieties, showing slight or no difference in reaction to leaf rust (*Puccinia triticina*, physiologic form 9) at different stages of growth or in diferent parts of the same plant. A.—Padui. B.—Resistant Fulcaster. C.—Kanred. D.—Michigan Wonder. Throughout the plate: *a*, Flag leaf of mature plant; *b*, lower leaf of same mature plant; *c*, prophyllum of seedling plant. Note that the lower leaves of Padui ,Kanred, and Michigan Wonder are slightly more resistant than the upper ones



Leaves of Mammoth Red wheat, showing difference in the reaction of leaves of the same plant to leaf rust (*Puccinia triticina*, physiologic form 9). A.—Flag leaf, resistant. B.—First leaf below flag leaf, susceptible. C.—Second leaf below flag leaf, susceptible. D.—Third leaf below flag leaf, somewhat resistant. E.—Prophyllum of seedling plant, susceptible

the third one below somewhat resistant. Thus in some varieties of wheat there seems to be little regularity about the location on the plant of leaves showing different rust reactions, aside from the fact that the flag leaf usually is the most resistant leaf of a plant. Resistant and susceptible leaves may be interspersed throughout the plant. Furthermore, plants often are observed in which differences in rust reaction occur in different parts of the same leaf, the tips of the leaves showing large normal uredinia, while the bases show only flecking or a few small uredinia surrounded by killed or necrotic areas. When this reaction was first observed it was attributed to a mixture of physiologic forms of rust, but later observations and careful testing of the rust culture proved it to be the typical reaction of the host. It should be kept in mind that the leaves of wheat grow in length from the base and that the oldest tissues therefore are near the tips. The situation then seems to be very similar to that noted above in which the older lower leaves produced uredinia, while the upper younger ones exhibited a high type of resistance.

DISCUSSION

These studies have shown that certain varieties of wheat may be highly susceptible to a given physiologic form of the leaf-rust fungus in the seedling stage and very resistant to the same form at heading time. Most of the varieties exhibiting that reaction in these experiments have shown also some resistance when grown under field conditions. At heading time in the field they usually are not so resistant as those varieties that have been resistant in all stages of growth in the greenhouse. Neither are they so susceptible as varieties that are highly susceptible in all stages of growth in the greenhouse. They often give a reaction intermediate between the two extremes. Such varieties usually exhibit a great deal of necrosis, especially on the upper leaves, but are not without considerable rust, principally on the lower leaves, even though they exhibit only a partial resistance. Varieties or hybrids of this nature usually are very obviously more desirable than completely susceptible ones.

The greenhouse experiments revealed reactions parallel in many respects to those observed in the field. In certain plants, susceptible as seedlings and resistant as mature plants, the uppermost leaves were the most resistant, while the lowermost often showed considerable rust. Similarly, in certain varieties, notably Mammoth Red, it was observed that the tips of the leaves were susceptible, while the younger tissues near the base showed a very high degree of resistance. It will be noted that in both cases the older tissues were rusted, while the younger ones showed signs of infection, but no rust developed. In the younger leaves metabolic processes are taking place rapidly, while the tissues of the older leaves are less active. It seems likely, therefore, that in such cases there is the formation or storing in the older tissues of certain compounds upon which the parasite lives and which are not formed or stored in the younger, more active tissues. Leaves of different ages and from different parts of plants showing this changing reaction are being collected for chemical analysis. If constant differences in the amounts of certain compounds can be demonstrated it may throw some light on the cause of resistance to leaf rust in wheat.

From the standpoint of the plant breeder, the facts brought out by these studies have considerable practical importance. They demonstrate that many promising resistant varieties and hybrids may be prematurely discarded if only seedlings are tested for rust resistance. It is true that these studies have shown that plants highly resistant in the seedling stage are resistant also in all other stages of growth. Such plants, therefore, are more desirable than those susceptible to rust at some time during their life history. But some desirable varieties contain no strains resistant to leaf rust at all stages of growth, while they do contain strains that, though susceptible as seedlings, are markedly resistant at heading time. These therefore are much more desirable than strains or varieties possessing no resistance to rust at any stage of growth, provided they are equal to susceptible strains in other respects.

It is very fortunate that plants of changing reaction to rust are more resistant in the heading than in the seedling stage, since heavy leaf-rust infection is more common at heading time than at any other stage of growth. Leaf rust usually does not become destructive until about the time the wheat plants begin to head. Then it appears in abundance and increases rapidly until the crop is mature. Varieties of wheat that develop resistance in their more advanced stages, therefore, are nearly as valuable in combating leaf rust as those that are resistant during their entire growth period.

No experiments have been conducted by the writers to ascertain whether varieties of wheat exhibit a changing reaction to stem rust or any other disease. It seems logical, however, that they may. It has been observed repeatedly at Manhattan and at other points in the vicinity that the lower leaves of wheat plants are heavily attacked by Septoria early in the spring at about the time the plants begin to joint, while the uppermost leaves seldom show signs of infection. Whether this is comparable to the conditions noted in the experiments reported in this paper is not known.

The phenomenon of increasing resistance to leaf rust with age was clearly demonstrated in certain varieties of wheat in these experiments. This change was observed in widely diverse types of wheat. It occurred in winter and spring, awned and awnless, hard and soft, and red-seeded and white-seeded varieties. The change from susceptibility in the seedling stage to resistance at heading time seems due to a difference in the chemistry or physiology of the young, active leaves, for the older leaves at the base of such plants often carry considerable rust.

SUMMARY

Experiments conducted in the greenhouse at the Kansas Agricultural Experiment Station have proved that certain varieties of wheat, very susceptible to leaf rust physiologic form 9 in the seedling stage, are highly resistant at heading time.

Of the varieties studied, Mammoth Red, Trumbull, Shepherd, Nittany, Marquis, Hungarian, Michikof, Russian, Goldcoin, Iobred, Rupert, and Red Clawson were definitely more resistant in the heading than in the seedling stage.

A number of other varieties changed only very slightly or not at all in their reaction to rust. Malakof, Bacska, Sibley, Banat, Brown

Bearded, and P721 were susceptible in all stages of growth, although some of them were slightly less susceptible in the heading stage.

Varieties resistant or rust free in the seedling stage are resistant at all stages of growth.

Those varieties that change in their reaction to leaf rust as they grow older show their highest resistance in the uppermost leaves. These leaves often show nothing but flecks, while those ranging downward from the top gradually become more susceptible until the lowermost leaves sometimes exhibit considerable infection.

Most of the varieties exhibiting this changing reaction to rust in the greenhouse also show considerable resistance to leaf rust under field conditions.

Many hybrid plants and selections also show a changing reaction to leaf rust in different stages of growth, it being especially noticeable in hybrid plants heterozygous with respect to their reaction to rust.

These experiments emphasize the fact that many promising varieties, strains, and hybrids may be discarded if only the seedling-stage rust test is used.

Latent resistance has been found in varieties representing the most important types of the common bread wheats. It has been observed in winter and spring, awned and awnless, hard and soft, and white-seeded and red-seeded varieties.

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THE FUNGICIDAL ACTION OF ULTRA-VIOLET RADIATION[1]

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INTRODUCTION

The bactericidal and fungicidal action of ultra-violet rays has been well recognized since the pioneer investigations of Downes and Blount[2] and of Marshall Ward.[3]

With the development of the quartz-inclosed mercury arc as a powerful source of ultra-violet radiation a number of practical applications have been made. Among these is the sterilization of various substances, for the most part liquids, through the killing action of the rays on bacteria and fungi.

The problem here investigated in a preliminary way is the surface disinfection of citrus fruits, which are opaque to the rays, with a view to reducing or retarding the development of decay, especially that caused by *Penicillium digitatum*.

EXPERIMENTAL PROCEDURE

The source of the ultra-violet radiation used in this investigation was a 110-volt quartz burner having a mercury cathode and a tungsten anode, normally operated on 320 watts (80 volts, 4 amperes). A water-cooled stage, consisting of a copper plate mounted in contact with a coil of thin-walled (0.1 mm.) copper tubing through which cold water was circulated, was used underneath exposed Petri dishes, to prevent overheating during prolonged irradiation. A detailed description of this apparatus and of the spectral-energy distribution of the mercury arc has been given in a previous paper.[4]

In making tests in Petri dishes the usual procedure developed after preliminary tests with other methods was to pour uninoculated nutrient agar, allow it to harden, and then apply the fungus spores to the surface as uniformly as possible either by barely flooding the surface with a few drops of a water suspension of the spores or by atomizing. In the latter case it was found advantageous to direct the nozzle of the atomizer upward and to hold the Petri dish in an inverted position some 30 cm. away so as to avoid the larger droplets. The exposures were made with the glass covers removed. Frequently a portion of

[1] Received for publication Oct. 1, 1928; issued February, 1929. This investigation was conducted as a cooperative project by the Bureau of Plant Industry of the U. S. Department of Agriculture and the Bureau of Standards of the U. S. Department of Commerce.

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the plate was screened with aluminum to serve as a check, or entirely unexposed plates inoculated similarly to the test plates were held as controls. The plates after exposure were kept at room temperature for several days until proper growth records could be made.

The spores of *Penicillium digitatum* and of *P. italicum* were usually taken from vigorously sporulating growth on recently rotted citrus fruits. No special effort was made to overcome the tendency of these spores to clump when mixed with water, because it was deemed important to conduct the tests with spore material such as might be found under practical working conditions in the citrus-packing industry. The spores of other fungi used in these tests were taken from freshly sporulating pure cultures.

PRELIMINARY TESTS WITH PENICILLIUM SPORES

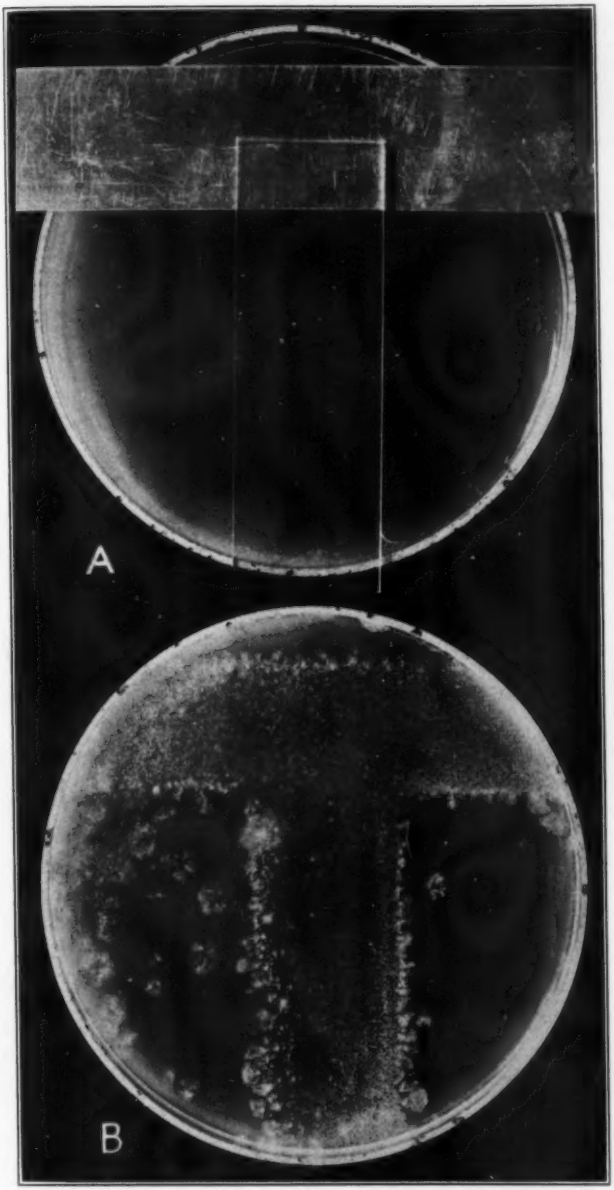
Shake cultures of *Penicillium digitatum* spores were made in cornmeal agar and poured to uniform depth in Petri dishes five hours before irradiation. Exposures at 7 inches from the lamp for 1, 3, 8, and 16 minutes resulted in reducing the after development of colonies to 25, 10, 5, and 2 per cent, respectively, as compared with 100 per cent developing in the check areas on the same plates screened with a 1-inch wide aluminum strip placed across the dish. (Pl. 1, A.) Microscopic examination showed that most of the growth in the irradiated portions came from clusters of spores where the outer ones doubtless had a protective screening effect upon the central ones of a group. Many of the surviving colonies were at the very margin of the Petri dish, where there may have been a protective influence on account of greater depth of agar or partial shading from the sides of the dish. The longer exposures developed a considerable heating effect, which may have had some disturbing influence. The area under the narrow aluminum strip, especially at its margins, however, must have been practically as warm as the adjacent portions of the irradiated area. The sharp killing effect at the boundary indicated that the heat developed was not sufficient, at least in itself, to kill. The growth under the aluminum strips was in every way comparable to that on entirely unexposed check plates of the same sowing. (Pl. 1, B.)

At the edges of thickly sown areas and where only a few colonies survive in the irradiated areas, the growth is very vigorous. Probably this is due mainly to freedom from crowding. It is also possible that the less than lethal dosages have a stimulating effect, such as has been noted by other investigators.[5]

A comparison was made of the effects of 5-minute exposures on poured plates of *Penicillium digitatum* spores distributed in cornmeal agar poured 24 hours, 6 hours, and 1 hour before irradiation. The sowing was at the rate of about 100 spores per square centimeter. The percentages of survival were estimated as 2, 5, and 10 per cent, respectively. This is taken to mean that growing mycelium is more readily killed than ungerminated spores.

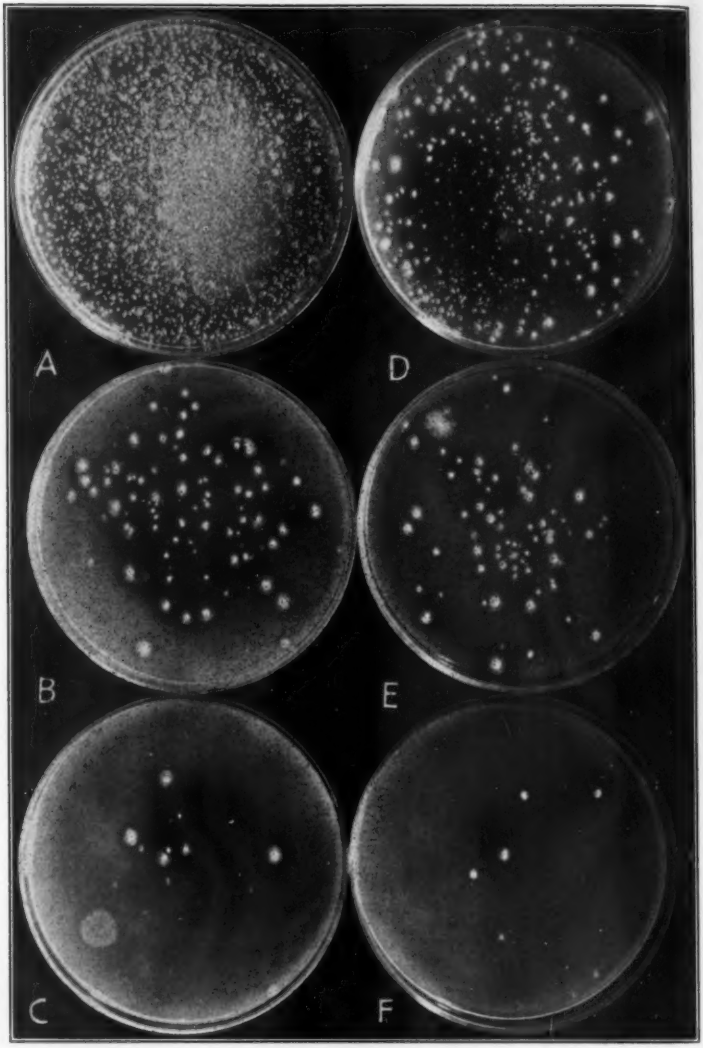
Since the agar itself has a protective influence, because of absorption of the rays, surface-smear sowings of *Penicillium digitatum* at

[5] DUFRÉNOY, J. ACTION DES RADIATIONS ULTRA-VIOLETTES SUR LES ZOOSPORES DE BLEPHAROSPORA CAMBIVORA PETRI ET DE PHYTOPHTHORA OMNIVORA PARASITICA. Rev. Path. Vég. et Ent. Agr. 12: [270]-272, illus. 1925.



A.—Petri dish showing the arrangement of an aluminum strip and a glass microscope slide used as screens during exposure to ultra-violet radiation

B.—Petri dish showing the practically equal effect of aluminum and glass screens in protecting *Penicillium digitatum* spores that were very thickly atomized over the nutrient agar on the plate. Exposure was for 3 minutes at 13 inches from the ultra-violet lamp, and the photograph was made 3 days later. Colonies at the edge of the protected areas and the scattered survivors in the irradiated areas are less crowded and show more extensive growth



AGAR PLATES ATOMIZED WITH A HEAVY SPORE SUSPENSION OF PENICILLIUM DIGITATUM

A.—Unexposed check.
B.—Irradiated discontinuously for a total of 15 seconds in three 5-second exposures with 5-second intervals.
C.—Irradiated discontinuously for a total of 45 seconds in nine 5-second exposures with 5-second intervals.
D, E, and F.—Irradiated 5, 15, and 45 seconds, respectively, with continuous exposures.
All plates were exposed 6 inches from the ultra-violet lamp and photographed three days after irradiation. Compare B and C with E and F.

the rate of about 20 spores per square centimeter were made on corn-meal agar plates 24 hours, 6 hours, and 1 hour before 5-minute exposure to the ultra-violet rays. The result was a complete killing of all spores. A comparison with the results outlined in the two preceding paragraphs indicates that the full intensity of the rays under the conditions indicated is sufficient to kill all spores of this species, whether in clumps or not, if the exposure is sufficiently prolonged.

In parallel tests with *Phomopsis citri*, the poured-plate sowings averaged about 50 spores per square centimeter and the surface smears about 20 spores per square centimeter. An irradiation of five minutes completely killed all spores for both methods of testing. Two obvious differences between Phomopsis and Penicillium spores might be factors in the more ready killing of the former. The walls of Phomopsis spores are thin and not highly colored, and these spores do not tend to cling together in clumps.

In another test, corn-meal agar plates were very heavily inoculated by flooding the surface with *Penicillium digitatum* spores. It was estimated from growth on check plates that there were 1,000,000 spores per plate. Exposures were made at 7 inches distance from the lamp for one-half, one, three, and five minutes. Three days later there was growth of 60, 82, 32, and 0 colonies, respectively, per plate. Similar plates exposed at a distance of 13 inches from the lamp and for the same periods of time showed later development of 85, 60, 35, and 8 colonies, respectively. The best survival shown in this series is less than 1 in 10,000 of the spores originally sown. The only complete kill obtained was with an exposure ten times as long and an intensity almost four times as great as was required to kill 9,999 out of 10,000 of the spores. In other words, the killing of absolutely all *P. digitatum* spores requires an extremely large amount of energy as compared with that required to kill 99.99 per cent of the spores. The main reason for the erratic survival of a relatively small number of spores after exposure for excessively long periods of irradiation is presumed to be the clumping of the Penicillium spores in such a way as to shade some from the rays.

PRELIMINARY TESTS WITH VARIOUS TYPES OF SPORES

A test was made on a number of miscellaneous fungi that happened to be readily available to determine differences in resistance to the abiotic effect of the ultra-violet rays. Water suspensions of spores taken from pure cultures were applied as counted drops on the surface of hardened corn-meal agar in Petri dishes, and the drops were spread over the surface as evenly as possible. Exposures of the uncovered dishes to the ultra-violet rays were made at a distance of 6 inches and for 1-minute and 4-minute periods. Unexposed plates were held as checks. Records were made after six or eight days. The results are given in Table 1.

Since killing was complete in one minute for most of these spores, a series of exposures shorter than one minute would have been required to bring out more completely the differences in resistivity. At the time of the above-mentioned test, plates of Hainesia spores were exposed for 15 seconds and for 30 seconds. These showed 4 surviving in the first instance and 1 in the second, out of an estimated sowing of 5,000 spores.

TABLE 1.—*Killing effect of ultra-violet radiation on various kinds of fungus spores*

Organism tested	Total number of colonies	Colonies surviving after exposure for—			
		One minute		Four minutes	
		Number	Per cent	Number	Per cent
Diplodia sp. (from lime fruit)	17	9	52.9	7	41.2
Helminthosporium sp. (from cranberry)	55	23	41.8	26	47.3
Alternaria sp. (from cranberry)	81	28	34.6	14	17.3
Cladosporium sp. (from cranberry)	400	62	15.5	18	4.5
Aspergillus niger	1,500	200	13.3	8	.5
Alternaria citri	600	60	10.0	13	2.2
Pestalozzia sp. (from cranberry)	1,000	20	2.0	22	2.2
Penicillium italicum	2,000	14	.7	2	.1
Chaetomella sp. (from cranberry)	600	2	.3	0	0
Hainesia sp.	5,000	1	Trace.	0	0
Colletotrichum gloesporioides	10,000	1	Trace.	0	0
Ceuthospora lunata	200	0	0	0	0
Rhizopus nigricans	1,000	0	0	0	0
Penicillium digitatum	6,000	0	0	0	0
Penicillium expansum	1,200	0	0	0	0
Phomopsis citri	800	0	0	0	0
Fusarium sp. (from orange fruit)	1,200	0	0	0	0
Botrytis sp. (from apple)	20	0	0	0	0
Glomerella rufomaculans	800	0	0	0	0
Phytophthora sp. (from orange fruit)	4	0	0	0	0
Monilia fructigena	200	0	0	0	0
Gloeosporium limetticolum	10	0	0	0	0
Anthostomella destruens	104	0	0	0	0
Guignardia sp. (from cranberry)	600	0	0	0	0
Melanconium sp. (from grape)	800	0	0	0	0
Acanthorhynchus vaccinii	900	0	0	0	0
Gloeosporium minus	1,000	0	0	0	0

It is to be noted that the more resistant fungi, as a group, are the ones having dark spore walls. Of the two common rots of citrus, *Penicillium italicum* seemed somewhat more resistant than *P. digitatum*, but it did not show an extraordinary percentage of survival.

The considerable difference in resistivity of different kinds of fungus spores suggests the possibility of using ultra-violet radiation in certain special cases to free mixed inoculum from undesirable contaminating organisms, in case these are more easily killed than the ones desired. Nonsporulating bacteria, as a rule, are more quickly killed than most fungus spores, and quick-germinating fungus spores might be killed by a dosage that would not destroy ungerminated types. Another laboratory use might be the surface disinfection of thin leaves or other delicate plant parts before culturing for organisms growing inside the tissues.

EFFECT OF INTERMITTENT EXPOSURES

The practical use of ultra-violet radiation for the surface disinfection of citrus fruits would involve intermittent exposure of any particular portion of the surface as the fruits are slowly revolved in passing on carriers under a battery of lamps. It is important to determine whether or not a series of short exposures would produce approximately the same killing effect as a single prolonged exposure of equal total duration. Corn-meal agar plates were atomized over the surface with a suspension of *Penicillium digitatum* spores and exposed at a distance of 6 inches from the lamp. A parallel series is shown for *Bacillus coli*, beef agar being used in this case. Observations were made three days after setting up. The results are given in Table 2.

TABLE 2.—*Abiotic action of ultra-violet rays on Penicillium digitatum and on Bacillus coli, showing comparative effect of continuous and discontinuous exposure*

Total duration of exposure	Method of exposure	Colonies surviving (per 1,000)	
		Penicillium digitatum	Bacillus coli
5 seconds	1 exposure of 5 seconds, continuous	93.3	2.7
10 seconds	1 exposure of 10 seconds, continuous	46.7	.8
	2 exposures of 5 seconds each, with 1 rest of 5 seconds	73.3	1.3
15 seconds	1 exposure of 15 seconds, continuous	29.3	1.3
	3 exposures of 5 seconds each, with 2 rests of 5 seconds	32.0	.7
20 seconds	1 exposure of 20 seconds, continuous	14.6	.2
	4 exposures of 5 seconds each, with 3 rests of 5 seconds	20.7	.2
30 seconds	1 exposure of 30 seconds, continuous	10.3	.2
	6 exposures of 5 seconds each, with 5 rests of 5 seconds	14.0	.4
45 seconds	1 exposure of 45 seconds, continuous	2.3	0
	9 exposures of 5 seconds each, with 8 rests of 5 seconds	3.3	0
	Average estimates for unexposed check plates	3,000	9,000
30 seconds	1 exposure of 30 seconds, continuous	3.3	8.3
	3 exposures of 10 seconds each, with 2 rests of 10 seconds	8.3	10
	Average estimates for unexposed check plates	1,200	6,000

While the continuous exposures gave a somewhat better kill than discontinuous ones of the same total duration, the differences do not seem to be great enough to be of importance in practical applications. (Pl. 2.) Wherever more than two short exposures were used the measure of the differences was less than 1 per cent of the organisms originally sown on the plates.

In a previous report[6] the writers have presented other experimental data substantiating this finding, based on experiments in which *Bacillus coli* was used as the test organism and in which the intensity of the radiation was greatly reduced so as to prolong the time required to kill, thus providing for a more accurate determination of the effect.

An attempt has been made to use the data presented in Table 2 for roughly evaluating what may be termed the killing effect upon *Penicillium digitatum* of each successively added 5-second period for both the continuous and the discontinuous applications. This has been done by estimating the percentage killed during each 5-minute period, each percentage being based on the number of spores living at the beginning of the particular period. Table 3 gives the results of these computations.

TABLE 3.—*Estimates of the killing effect upon Penicillium digitatum of successively added 5-second periods of exposure to ultra-violet rays continuously and discontinuously applied*

Periods	Per cent killed during each period of—	
	Continuous exposure	Discontinuous exposure
First 5 seconds of exposure	91	----
Second 5 seconds of exposure	50	21
Third 5 seconds of exposure	37	56
Fourth 5 seconds of exposure	50	35
Fifth and sixth 5 seconds of exposure, average	14	16
Seventh, eighth, and ninth 5 seconds of exposure, average	26	25

[6] COBLENTZ, W. W., and FULTON, H. R. Op. cit.

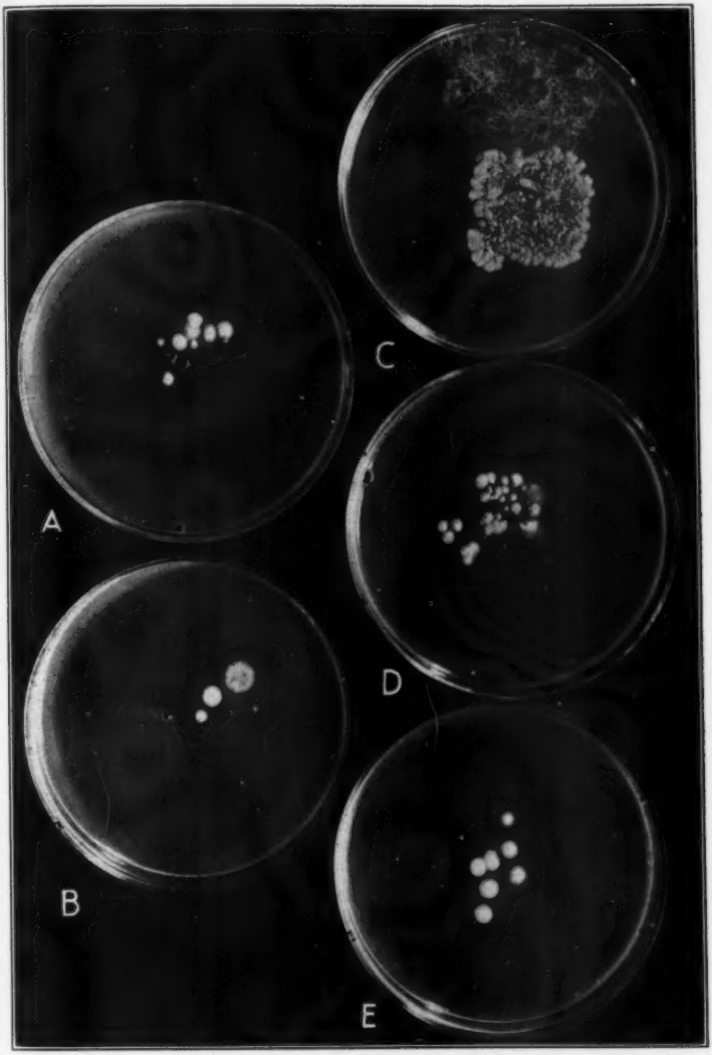
There is a general although irregular tendency for the killing effect to decrease for the later periods of exposure. It is probable that accidental screening of spores from the rays rather than wide difference in inherent protoplasmic resistivity accounts for the difficulty in killing absolutely all spores. The discontinuous applications average about the same as the continuous ones, when the third and succeeding applications are considered.

DISINFECTION OF THE SURFACE OF ORANGES

The possible thoroughness with which the surface of oranges can actually be disinfected by the use of ultra-violet radiation was tested as follows: The fruits were dipped in a heavy suspension of *Penicillium digitatum* spores and dried; six hours later they were exposed for one-half, one, two, and four minutes to ultra-violet radiation at a distance of 13 inches; afterwards the previously radiated portion was gently rolled over very stiff nutrient agar solidified in Petri dishes, and these were incubated three days. From fruits exposed four minutes an average of 7 colonies per fruit developed; from those exposed two minutes, an average of 24 colonies; from those exposed one minute, an average of 21 colonies; from those exposed one-half minute, an average of 40 colonies; and from the unexposed checks, an average of 1,300 colonies. One series of plates from this test is shown in Plate 3.

Another method to test the sterility of the surface after irradiation was the following: The oranges were dipped in a suspension of *Penicillium digitatum* spores and dried; immediately afterwards they were exposed to the ultra-violet rays in five positions—two polar and three equatorial—each exposure being for 10 seconds; half the surface of each fruit was swabbed over with moist sterile cotton, the swabs were shaken up in 10 c. c. of sterile water, dilutions of 1/10 and 1/100 were made from this to nutrient agar, and plated. As a result of this procedure it was estimated that an average of 130,000 Penicillium spores were swabbed from half the surface of a nonirradiated fruit, and that the overlapping exposures of 10 seconds each on five different aspects of the fruit destroyed about 90 per cent of the spores.

In another similar test the oranges were divided into four lots. The fruits in one lot were given 6 overlapping exposures—2 polar and 4 equatorial—each exposure being for 30 seconds, at 13 inches from the lamp; in another lot the fruits were given 2 discontinuous exposures of 30 seconds each for each of the six aspects; in a third lot the fruits were given 3 discontinuous exposures of 30 seconds each; and the fourth lot was not exposed to the ultra-violet rays. By the swabbing method it was estimated that the fruit given a single set of 30-second exposures had about 400 Penicillium spores remaining viable; those given the double exposure, about 100 viable spores; those given the triple exposure, amounting to a total of 1½ minutes on each aspect of the fruit, about 10 viable spores; whereas the nonirradiated checks had about 40,000 viable spores. Expressed otherwise, the percentages killed by the three methods, based on these estimates, would be 99.000, 99.750, and 99.975, respectively. The more prolonged series of exposures, amounting to a total of 9 minutes for the fruit as a whole, killed approximately 3,999 out of 4,000 spores. It must be borne in mind that some of the few surviving spores might



Agar plates over the central portions of which oranges were rolled after having been dipped in a suspension of *Penicillium digitatum* spores, dried, and variously irradiated at a distance of 13 inches from the ultra-violet lamp. Photographed after incubation of the plates for three days: C, From an orange that was not irradiated; D, E, A, and B, from oranges irradiated for ½, 1, 2, and 4 minutes, respectively

be in advantageous positions for the starting of infection, such as in a wound crevice. One living spore so placed, under favoring conditions, might initiate rot just as certainly as though the fruit had not received treatment. There is no continuing retarding effect, as may occur from residues of a chemical disinfectant, and no protection from later contamination.

HOLDING TESTS WITH ORANGES

Several practical tests of oranges were made to see how much reduction in development of actual rot could be brought about by exposing the surface of the fruit to ultra-violet radiations. In one of these tests the oranges (31 in each lot) were dipped in a suspension of *Penicillium digitatum* spores and were wounded by puncturing 100 times each. One lot was irradiated on six faces for one minute each at a distance of 13 inches from the lamp, another lot on six faces for one-half minute each, and a third check lot was not irradiated. At the end of three days the three lots had developed 13, 19, and 26 blue-mold rots, respectively; at the end of six days the totals for blue-mold rot were 31, 28, and 33, out of the 36 originally inoculated in each test. Swabbing tests of the surface of some of these fruits indicated an average of about 50, 48, and 1,000 viable spores per fruit for the three lots, respectively, which would mean about 95 per cent killing of the spores.

In another test, two lots of 32 fruits each were inoculated and punctured as described above. One lot was exposed to ultra-violet radiation for 10 seconds each on five faces at a distance of 9 inches from the lamp, while the other lot was not treated with the rays. After six days holding at room temperature the blue-mold rots were 14 and 13, respectively; and after nine days they were 26 and 30, respectively, out of the 32 fruits originally in the test.

In still another test, 200 fruits were inoculated and wounded. Half of them were irradiated for 10 seconds on each of six faces at a distance of 13 inches from the lamp, and half were not irradiated. The progressive development of total blue-mold rot is indicated as follows: Third day, 9 per cent for the treated and 34 per cent for the untreated; sixth day, 62 and 86 per cent; ninth day, 78 and 92 per cent; twelfth day, 83 and 93 per cent; fifteenth day, 85 and 94 per cent.

No appreciable effect on the development of stem-end rot was noted in any of the tests. In two instances special tests for the effect in checking stem-end rot gave negative results. There was no evidence of any deleterious effects to the fruit in any of the tests as a result of the exposures to ultra-violet rays.

While there is some evidence that the ultra-violet treatment retards the development of rot during the first few days of holding, the final amounts of rot during a usual marketing period are very much the same in the treated and untreated lots in all tests. The conditions of these tests were purposely made very severe in order to give every fruit a chance to become infected except as the ultra-violet treatment might prevent such infection. In commercial practice only a small percentage of the fruit ordinarily would be exposed to such severe infection. These fruits, however, are the ones that require protection, which should be adequate to insure their preservation under very severe conditions.

RELATIVE FUNGICIDAL ACTION OF DIFFERENT WAVE LENGTHS OF LIGHT

It has been seen that, taking the ultra-violet radiation as a whole, the fungicidal effect varies with the time of exposure and with the intensity of the light. The latter varies inversely as the square of the distance from the source and may be affected to some degree by imperfect transmission through media intervening between the source and the fungus material to be acted upon. In the investigation of bactericidal action previously referred to, the writers have tested the spectral range of abiotic action on *Bacillus coli* by using mica or glass screens that transmit the different regions of the ultra-violet spectrum in different degrees. It was found that germicidal action for this organism is produced by ultra-violet radiation extending from the shortest wave lengths tested (about 240 μ) to and including 365 μ. The action probably extends beyond this upper limit, but in very weak degree. The shortest rays have the most violent lethal action, which decreases in intensity with increase in wave length. By the same method of using mica or glass screens, preliminary tests with fungus spores bear out the same general conclusion, namely, that different wave-length components have different killing power, which increases as wave length decreases over a wide range, the lethal action beginning somewhere about 365 μ as an upper limit.

As a result of absorption in the atmosphere, sunlight at the earth's surface has an ultra-violet component with wave length extending to about 313 μ as the lower limit. This corresponds to the upper and less-effective range of ultra-violet radiation from a mercury-arc lamp. Consequently exposures to sunlight to be lethal for fungi must be much more prolonged than in the case of radiation, as from the mercury arc, which includes quick-killing shorter wave lengths down to 240 μ. Ordinary glass intercepts some of the shorter wave-length rays reaching the earth in sunlight and reduces its fungicidal action. A test was made by exposing spores of *Penicillium digitatum* and of *Phomopsis citri* for 16 minutes to radiation from a glass-inclosed mercury-arc lamp. No killing effect could be determined. Furthermore, the intervention of ordinary thin microscope slides during exposure to emanations from the quartz-mercury lamp afforded complete protection for periods of exposure that produced a high percentage of killing when the light was unobstructed.

CONCLUSIONS

Ultra-violet rays are capable of killing fungus spores, in most cases after a comparatively brief exposure. Some types of spores require very much longer exposures than others for killing, probably on account of the difficulty in ray penetration of the spore walls due to their protective coloration or to their composition. The outer spores of clumps have a protective shading effect on those in the center of the mass, and the latter survive exposures several times longer than is requisite to kill the single detached spores. Growing mycelium is more easily killed than are ungerminated spores. Mycelium may penetrate a substance that is opaque to the rays and thus escape the killing effect.

The fungicidal effectiveness of ultra-violet treatment depends on the intensity of the radiation and on the wave lengths of the component rays. The shorter the wave length the more lethal the effect with a given intensity. The killing effect is cumulative, several short intermittent exposures having almost the effect of one continuous exposure of the same total time. The ultra-violet rays, especially those of shorter length, have a very limited penetrating power. The yellow or orange coloration of mature citrus fruits acts as a very effective protective screen to prevent penetration by the rays. Irregularities in the fruit surface or the presence of foreign particles would tend to have a shading effect on some of the spores.

The spores that are in the best position actually to start decay are the ones that are in punctures or other breaks in the skin, and these are most likely to be protected from full exposure to the rays. Mycelium that has penetrated the citrus tissue to any extent could not be reached by the rays. The ultra-violet treatment does not afford protection from later contamination with blue-mold spores. In addition to these limitations there is the difficulty and expense of making a mechanical installation that will insure complete and sufficiently prolonged exposure of all parts of the fruit under packing-house conditions. On the whole it would seem that other methods of treating citrus fruit to prevent blue-mold rot are more feasible and economical at the present time than the use of ultra-violet radiation as it is now capable of being produced and applied commercially.

Ultra-violet radiation has been successfully used for the disinfection of water. Its limitations here are the expense and the comparatively small volume of water that can be treated in a given time. It is possible that the wash water used in citrus packing houses might be disinfected by this means, but under ordinary circumstances it would be less expensive to renew the wash water than to incur the relatively high cost of using ultra-violet radiation for the purpose of keeping it sterile.

SUMMARY

Spores of 27 miscellaneous species of fungi were exposed for one minute to ultra-violet radiations from a quartz-inclosed mercury-tungsten arc at a distance of 6 inches. There was complete killing of the spores of 16 species, a survival of less than 1 per cent in the case of 4 species, and survival ranging from 2 to 53 per cent for the remaining 7 species. Mycelium from germinated spores was more easily killed than the resting spores.

The killing effect of ultra-violet rays is increased with the lengthening of the time of action and also with the increase in the intensity of the radiation. Decrease in the distance from the source of radiation results in increase in intensity and in consequent killing effect. An exposure of 5 seconds at 6 inches was sufficient to kill at the rate of 907 out of 1,000 spores of *Penicillium digitatum*. With an exposure of 45 seconds the rate of killing was 998 out of 1,000. It is supposed that partial shading of some of the spores accounts for the accidental survival of a few.

A series of short exposures has almost the same effect in a cumulative way as one continuous exposure of equal total duration. Penicillium spores on the surface of citrus fruits can be killed in large proportions by intermittent short exposures to ultra-violet rays.

The ultra-violet rays in the dosages used did not cause any apparent injurious effect on the oranges.

In holding tests, decay of inoculated oranges was only moderately retarded by applications of ultra-violet rays that seem to reach the limit of economical practice under present conditions for time of exposure and distance from lamps.

The principal physical limitations to the use of ultra-violet rays for complete disinfection of fruits seem to be their inability to penetrate much if any below the surface, so as to destroy mycelium that may have started infection already, and the accidental shading effects that favor the survival of some of the contaminating spores.

The shorter wave-length components of the ultra-violet spectrum have the greatest fungicidal action. The longer wave lengths of this spectrum, such as are components of sunlight, have relatively weak fungicidal power, and require greatly prolonged exposures for the killing effect.

A SEROLOGICAL RANKING OF SOME WHEAT HYBRIDS AS AN AID IN SELECTING FOR CERTAIN GENETIC CHARACTERS[1]

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INTRODUCTION

The complex of a species concept includes not only morphological and physiological but also chemical factors. Although the characteristics which help to distinguish species may include differences in lipoid, carbohydrate, mineral, and protein content, it is commonly believed that the determinative factors of the species are to be found within the protein constituents of the individual. This protein difference characterizing a species can be studied advantageously by immunological reactions. Nuttall (*15*),[2] Uhlenhuth (*21*), and others, using this technic, have found that the protein characterizes the species in the animal kingdom. Wells (*22*) and others in the United States and Zade (*25*) and more recently Mez (*12*) in Germany have found that the species of plants are characterized by the specificity of their protein. In fact, Mez has attempted a complete botanical classification based on protein specificity. Nuttall's zoological and Mez's botanical classification, both based on protein specificity, confirm in the main the classifications based on morphology. This is to be expected, since protein differences are almost undoubtedly responsible for morphological as well as physiological differences.

Although the first serological investigations of species were made as long ago as 1900, it is still quite commonly believed by investigators that the application of this method is limited to genus and species differentiation. Zade states that varieties can be differentiated by this technique, in consequence of which his classification based on serological differentiation is limited to the species. However, a refinement of the technique of the precipitin test makes it possible to distinguish between varieties within the species. Since this is true, the precipitin test should be of value to the geneticist in selecting hybrids for desired characteristics. The data in this paper are offered as experimental evidence of the value of this test to the geneticist in selecting hybrids for desired characters.

Breeding, which is the application of the laws of genetics to the production of new individuals, always has rested on selection and probably always will continue to do so (*6*, *7*). The principal influence of the science of genetics has been to shift the attention in selection from the phenotype to the genotype. When the characters in question are visible, such as color, presence or absence of awns, etc., the work of selection is relatively simple. The problem becomes

[1] Received for publication Sept. 13, 1928; issued February, 1929.
[2] Reference is made by number (italic) to "Literature cited," p. 180.

more difficult when the character sought is invisible, such as the resistance to disease (*1*, *3*, *4*, *8*). The empirical method now used is laborious and time consuming. It is obvious that a more rapid method of measuring the invisible characters of a plant or animal would be highly desirable. Assuming that these characters are bound up in specific proteins, the serological method which identifies the latter offers a valuable approach to the problem.

This article reports an attempt to apply the serological method to a determination of the relationships existing between certain well-known hybrid wheats. These hybrids have been selected by breeders for characters such as stem-rust resistance, yield, hardness, etc., which particularly recommend them.

Four varieties of common and one of durum wheat were chosen for study. The durum wheats have 14 haploid chromosomes (*10*); the vulgare wheats have 21. Percival (*19*) states that the durums, as a class, show greater resistance to disease than do the common wheats. Mindum, the durum used, is not, however, a particularly resistant member of this group. Mindum is a selection from Hedgerow (*5*). Marquis is the result of a cross between Hard Red Calcutta and Red Fife. Hard Federation is a selection from Federation. 1656–81 is a selection from a Kota-Marquis cross produced at the North Dakota Agricultural Experiment Station. Hope, a highly resistant wheat, is the result of a cross between Yaroslav emmer, which has 14 haploid chromosomes, and Marquis, which has 21.

According to L. R. Waldron, of the department of agronomy at the North Dakota Agricultural College, the varieties of common wheat used in the work rank, in the order of resistance to stem rust, as follows: Hope, 1656–81, Marquis, and Hard Federation.

METHODS OF EXPERIMENTATION

EXTRACTING GLOBULINS

Three different methods for extracting globulins were tried (*17*, *20*).

SERIES A

Five hundred grams of wheat was ground five times, using three different sizes of burrs. The flour was digested for 12 hours at 37° C., using 10 c. c. of 8 per cent NaCl per gram of flour. The clear liquid was decanted and then filtered with a suction filter to remove any particles not in solution. Globulin precipitation was effected by half saturating the filtrate with $(NH)_2SO_4$. The precipitate was allowed to form overnight at 37° C. The supernatant liquid was discarded and the precipitate redissolved in distilled water, enough salt remaining in the precipitate to dissolve the globulin. The material was then centrifuged to remove any particles not in solution. The globulin was precipitated from the solution to which thymol had been added to prevent bacterial action during dialysis. A continuous dialyzer, like that described by Hanke (*9*) was used. Complete dialysis required about 18 days. The globulin was then purified by redissolving it in 0.85 per cent NaCl and reprecipitating by dialysis.

This method of dialysis consumed so much time that there was a possibility of denaturing the protein. To avoid this danger a modi-

fied form of the electrodialyzer described by Locke and Hirsch (*11*) was used. Instead of the paper shells which they suggest, fish-skin membranes were employed. Dialysis by this method required only two to six hours, depending upon the completeness desired.

Series B

A second series of globulin separations was prepared by the same method as before except that the globulin solution was electrodialyzed. The dialyzer described above was used. Dialysis required from two to six hours. The globulin fraction thus obtained was further purified by redissolving in 0.85 per cent NaCl, and reprecipitating by electrodialysis.

Series C and C′

A third and a fourth series of globulin were prepared. For the third series C, wheat was ground and treated with NaCl as before. The material was digested first for 8 hours in a rotary ball mill at room temperature, and then for 12 hours, at 37° C. The clear liquid was removed by decanting and filtering. The NaCl extract was dialyzed. The precipitate obtained was further purified by redissolving in 0.85 per cent NaCl, and the globulin was reprecipitated by electrodialysis. After three hours of dialysis the precipitate was collected and dried at 37°. The yield was about 2.3 gm. or 0.46 per cent globulin. This precipitate, corresponding to the so-called "globulin," was used as antigen in all of the experiments herein reported. The precipitate used for C′ was obtained from two varieties, Hope and 1656–81, by diluting the original highly saline extract to five times its volume with distilled water, just previous to dialysis. The yield was insignificant (0.01 gm.) and showed that not much globulin was being missed by the other procedure. To check the identity of this protein precipitate it was used as antigen and titrated in a series of precipitin tests with antisera prepared with globulin from series A, B, and C. This is called series C′. Tables 6 and 7 present data obtained from these comparisons.

Reaction

The proteins, as has been observed (*2*, *14*), became increasingly insoluble with age and manipulation. The hydrogen-ion concentration ranged from 5.2 to 5.8. All pH determinations were made by the colorimetric method; methyl red and brom cresol purple were used as indicators. The proteins apparently became increasingly acid on standing in normal salt solution, thus requiring frequent adjustment with NaOH to maintain solubility.

Preparation of Antigen

The globulins were dried, weighed, and then pulverized in a sterile mortar. Enough 0.85 per cent NaCl solution was added to give a globulin concentration of 0.002 gm. per cubic centimeter. This antigen solution was diluted to five times its volume before it was used in the titrations, giving a concentration of 0.0004 gm. per cubic centimeter.

PRECIPITIN TESTS

SENSITIZATION

Five 6-months-old rabbits that had been fed on a wheat-free ration were selected for sensitization. The dosage used was 0.002 gm. of antigen in a volume of 1 c. c. of normal saline solution. The rabbits were injected intraperitoneally as follows:

Series 1.—Four injections were made at 5-day intervals. Animals were bled on the eleventh day after the last injection.

Series 2 and 3.—Two injections were made at 5-day intervals. Animals were bled on the eleventh day after the last injection.

The animals were starved for a period of 30 hours before they were bled. About 15 c. c. of blood was drawn from the ear. A period of four days elapsed between each bleeding and the subsequent injection of antigen. The blood was stored at ice-box temperature for 12 to 15 hours so as to allow the normal clot to form. It was then centrifuged at a low rate of speed, the clear serum thus obtained being stored at ice-box temperature, except when actually in use.

Two normal rabbits were made as checks.

Check experiments were used as follows:

Normal serum × the various antigen	No precipitate.
Normal serum × the various sera	Do.
Antigen × antigen	Do.
Serum × serum	Do.

READINGS

The readings were made independently by several coworkers at 4, 8, and 18 hour intervals. The observed variation was insignificant. The average of all readings taken is reported. The amount of precipitate obtained in homologous titration was used as a standard in the heterologous titrations. A numerical reading of 10 corresponds to a reading of ++++, which is frequently used. At a serum dilution of about 1:80 the greatest amount of precipitate was obtained. The prezone (*13*, *16*, *18*) phenomenon was observed in the lower dilutions. The serological relationships of the different varieties of wheat are shown in Tables 1 to 5.

TABLE 1.—*Comparison of homologous and heterologous specificities, using Mindum antiserum*

[A numerical reading of 10 corresponds to ++++]

Antigen	Series[1]	Numerical readings at serum dilutions of—						Total[2]	Per cent	Rank
		1:20	1:40	1:80	1:100	1:200	1:400			
Mindum	1	3	6	6	6	3	3	18	100	1
	2	5	6	6	5	4	3	18		
	3	1	5	6	3	2	2	13		
Hope	1	3	3	3	2	1	0	6	39	2
	2	2	3	2	2	1	1	6		
	3	6	6	4	1	1	1	7		
1656–81	1	2	2	3	2	0	0	5	37	3
	2	2	3	3	2	1	0	6		
	3	3	2	3	2	2	0	7		
Marquis	1	3	2	1	1	0	0	2	27	4
	2	1	2	2	2	1	0	5		
	3	2	2	2	2	2	0	6		
Hard Federation	1	0	2	1	1	0	0	2	14	5
	2	1	1	2	1	0	0	3		
	3	2	1	1	1	0	0	2		

[1] The work was repeated twice. The numerals 1, 2, and 3 represent in each case the series of sensitization. Attention is called to the fact that the same animals were used each time.

[2] The last four dilutions of each series were totaled. A total of the three homologous series was chosen as a basis of comparison with the heterologous titrations.

TABLE 2.—*Comparison of homologous and heterologous specificities, using Hard Federation antiserum* [1]

[A numerical reading of 10 corresponds to ++++]

Antigen	Series	Numerical readings at serum dilutions of—						Total [1]	Per cent	Rank
		1:20	1:40	1:80	1:100	1:200	1:400			
Hard Federation	1	6	6	6	7	6	3	22	100	1
	2	5	8	8	7	5	4	24		
	3	9	8	7	7	5	4	23		
Marquis	1	6	6	4	5	4	3	16	64	2
	2	0	2	3	3	3	3	12		
	3	7	7	6	5	4	1	16		
1656–81	1	3	5	5	4	3	2	14	49	3
	2	1	1	2	2	3	2	9		
	3	3	7	4	4	2	1	11		
Hope	1	3	3	4	3	2	1	10	30	4
	2	1	2	1	2	1	0	4		
	3	4	4	3	2	1	1	7		
Mindum	1	1	3	3	1	0	0	4	19	5
	2	3	4	2	2	1	0	5		
	3	0	0	1	1	1	4	1		

[1] See footnotes to Table 1.

TABLE 3.—*Comparison of homologous and heterologous specificities, using Hope antiserum* [1]

[A numerical reading of 10 corresponds to ++++]

Antigen	Series	Numerical readings at serum dilutions of—						Total [1]	Per cent	Rank
		1:20	1:40	1:80	1:100	1:200	1:400			
Hope	1	9	9	9	6	3	3	21	100	1
	2	8	8	10	8	6	6	30		
	3	8	0	6	6	5	3	20		
1656–81	1	7	7	5	5	4	2	16	63	2
	2	4	6	4	4	3	2	13		
	3	8	6	5	5	3	3	16		
Marquis	1	5	7	6	3	2	1	12	54	3
	2	2	3	3	2	2	2	9		
	3	9	8	5	4	4	4	17		
Mindum	1	1	1	3	4	2	0	9	27	4
	2	4	3	2	2	2	0	6		
	3	1	1	1	1	1	1	4		
Hard Federation	1	3	3	4	3	2	1	10	25	5
	2	1	1	1	1	1	1	4		
	3	5	2	1	1	1	1	4		

[1] See footnotes to Table 1.

TABLE 4.—*Comparison of homologous and heterologous specificities using Marquis antiserum* [1]

[A numerical reading of 10 corresponds to ++++]

Antigen	Series	Numerical readings at serum dilutions of—						Total [1]	Per cent	Rank
		1:20	1:40	1:80	1:100	1:200	1:400			
Marquis	1	1	6	8	7	5	4	24	100	1
	2	6	7	8	7	6	4	25		
	3	6	6	3	2	3	3	11		
1656–81	1	6	4	5	4	4	3	16	72	2
	2	8	8	6	5	4	3	18		
	3	5	5	3	3	2	1	9		
Hope	1	6	6	6	3	2	1	12	67	3
	2	8	8	6	5	4	4	19		
	3	5	3	3	3	2	1	9		
Hard Federation	1	6	6	3	3	2	1	9	53	4
	2	7	6	5	4	4	3	16		
	3	4	3	2	2	2	1	7		
Mindum	1	6	3	3	2	1	0	6	32	5
	2	4	3	3	3	2	2	10		
	3	1	2	1	1	1	0	3		

[1] See footnotes to Table 1.

TABLE 5.—*Comparison of homologous and heterologous specificities using 1656–81 antiserum* [1]

[A numerical reading of 10 corresponds to ++++]

Antigen	Series	Numerical readings at serum dilutions of—						Total [1]	Per cent	Rank
		1:20	1:40	1:80	1:100	1:200	1:400			
1656–81	1	9	9	9	8	5	5	27	100	1
	2	8	8	7	6	6	5	24		
	3	10	9	8	5	4	2	19		
Hope	1	9	9	7	6	4	2	19	83	2
	2	8	8	7	8	5	3	23		
	3	9	7	6	5	3	2	16		
Marquis	1	9	6	3	3	3	3	12	63	3
	2	3	3	4	5	4	3	16		
	3	9	7	6	5	3	2	16		
Hard Federation	1	3	6	3	3	2	1	9	37	4
	2	5	5	3	2	2	1	8		
	3	6	5	4	3	1	1	9		
Mindum	1	3	3	3	1	1	0	5	23	5
	2	3	3	2	2	2	0	6		
	3	2	3	3	1	1	0	5		

[1] See footnotes to Table 1.

ABSORPTION TESTS

The serological ranking of the several globulins with reference to each homologous globulin-antigen in turn is based on the assumption that the volume of precipitate is directly proportional to the genetic relationship. In precipitin technique it has been demonstrated that the bulk of the precipitate formed is from the specific serum used but in association with the specific portion of the antigen. This being true, it should be possible to treat an antigen with a heterologous serum and precipitate the common protein fraction or characters; then to remove the clear liquid, and to add to it another heterologous serum. This latter should precipitate those proteins, factors or characters in the antigen specific to it but which have not been removed by the preceding serums.

One experimental difficulty, that of obtaining the proper concentration relationship between the antigen and the antiserum, presents itself. To saturate the antigen completely for the specific character concerned without adding an excess of serum requires fine adjustment. It has been shown that an excess of serum interferes with the formation of or redissolves the precipitate.

In Figures 1 to 5, which follow, the data from the absorption experiments are incorporated. Each of the five wheat globulins was put in saline solution at a concentration somewhat greater than that required for optimum precipitation, i. e., about 1:40. To this antigen solution was then added sufficient antiserum in concentrated form to yield a serum concentration of approximately 1:80. The mixture was placed in the incubators at 37° C. for four to eight hours to obtain the greatest amount of precipitate. Each serum-antigen mixture was prepared in triplicate to insure accuracy of observation. After the first addition of serum to the antigen, and the formation of precipitate the volume of precipitate was estimated, the tubes were centrifuged at 3,000 revolutions per minute, the clear fluid was added to more antigen solution and incubated for further precipitate formation. The new precipitate, if any formed, was estimated and added to that formed previously for a new total quantity. The total precipitate which formed upon complete saturation of an optimum antigen solution (about 1:40) with an optimum serum concentration (about 1:40 to 1:80) of a homologous serum was taken as a basis of precipitin volume comparison, on a basis of 10. The figures illustrate graphically what is expressed numerically in the Tables 6 and 7.

TABLE 6.—*A comparison of the effect of the methods of globulin extraction on specificity, using 1656–81 antiserum (Series C)*

Antigen	Series [1]	Numerical readings at dilutions of—						Total [2]	Per cent
		1:20	1:40	1:80	1:100	1:200	1:400		
1656-81	C	10	9	8	5	4	1	18	100
	C′	4	2	3	3	4	1	11	61
	B	0	3	0	3	2	1	6	33
Hope	A	3	2	3	3	2	1	9	50
	C′	9	7	6	5	3	2	16	89
	C	2	2	3	2	1	1	7	39
Marquis	B	2	0	1	2	2	0	5	28
	C	9	7	6	5	3	2	16	89

[1] Series A: Globulin extracted by precipitating with $(NH_4)_2SO_4$ and dialyzed using the continuous dialyzer described by Hanke (*9*). Time required, 18–21 days. Since the time required was so great, this method was discontinued after making one separation.
Series B: The globulin was extracted by using $(NH_4)_2SO_4$ and electrodialysis.
Series C: The globulin was extracted by electrodialysis of the NaCl extract.
Series C′: The liquid portion remaining after dialyzing C was diluted five times and further dialyzed for two days. A small precipitate was obtained.
[2] The last four dilutions of each series were totaled.

TABLE 7.—*A comparison of the effect of the methods of globulin extraction on specificity, using Hope antiserum (Series C)* [1]

Antigen	Series	Numerical readings at dilutions of—						Total [1]	Per cent
		1:20	1:40	1:80	1:100	1:200	1:400		
Hope	C	8	9	6	6	5	3	20	100
	C′	3	2	3	5	3	2	13	65
Marquis	C	9	8	5	4	4	4	17	85
	C′	0	0	0	2	3	0	5	25
1656-81	C	8	6	5	5	3	3	16	80
	C′	2	2	2	5	3	2	12	60
	B	2	0	0	3	3	0	6	30
	A	3	3	3	4	3	1	11	55

[1] See footnotes to Table 6.

The data on comparisons of specificity of globulins obtained by the several variations in method and presented in Tables 6 and 7 are not complete because the tests were limited in number by the supply of globulins available for the purpose. Globulins of series C, extracted by electrodialysis of sodium chloride extracts, were the most specific. Globulins from series C′ were next in degree of specificity. This is explainable on the basis of time; i. e., the time required for dialysis in the methods used for series A and B was so long as to permit denaturing of the proteins.

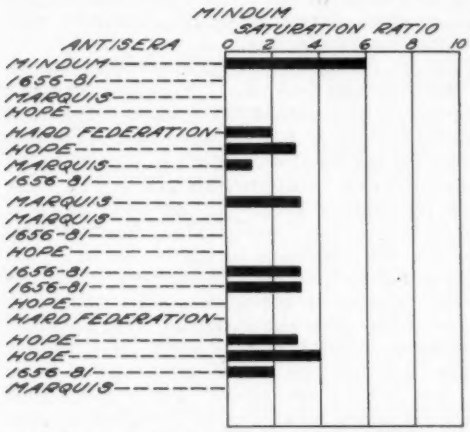

FIG. 1.—Results of absorption experiments using Mindum as the absorbing antigen. Antisera in order of application, using 1:80 dilution only: Mindum (6); 1656-81 (0); Marquis (0); Hope (0). Hard Federation (2); Hope (3); Marquis (1); 1656-81 (0). Marquis (3); Marquis (0); 1656-81 (0); Hope (0). 1656-81 (3); 1656-81 (3); Hope (0); Hard Federation (0). Hope (3); Hope (4); 1656-81 (2); Marquis (0)

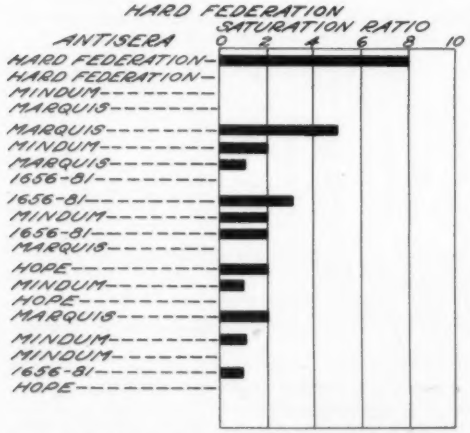

FIG. 2.—Results of absorption experiments using Hard Federation as absorbing antigen. Antisera in order of application, using 1:80 dilution only: Hard Federation (8); Hard Federation (0); Mindum (0); Marquis (0). Marquis (5); Mindum (2); Marquis (1); 1656-81 (0). 1656-81 (3); Mindum (2); 1656-81 (2); Marquis (0). Hope (2); Mindum (1); Hope (0); Marquis (2). Mindum (1); Mindum (0); 1656-81 (1); Hope (0)

The apparent inconsistency of the rank occupied by Mindum antigen in cross titrations with Hope, Marquis, and 1656–81 antisera may be explained on the basis of antigen complexity. This is further exemplified in the absorption tests.

Absorption of the antigen by the homologous serum removed the precipitable fractions in the former so that no precipitate was obtained upon the subsequent addition of heterologous sera. Figure 3 shows an apparent exception in that the addition of 1656–81 serum after homologous absorption, using Hope antigen and antiserum, gave an additional precipitate. This was due, no doubt, to the fact that Hope antigen was not at saturation at this dilution. (Table 3.) At dilutions 1:20 and 1.40 more precipitates were obtained. This shows the necessity for as well as the difficulty of securing complete saturation without an excess.

A repetition of the work using several serum dilutions might avoid this technical difficulty.

The type of interpretation applicable to Figures 1 to 5 may be illustrated thus in the case of Figure 1.

(*a*) The homologous antiserum completely absorbs Mindum antigen.

(*b*) A study of the results of applying to Mindum antigen the several heterologous antisera in the various possible orders show that a greater portion of Mindum antigen is precipitated by the Hope-1656–81-Marquis group of antisera than by Hard Federation antiserum, and relatively more by Hope antiserum than by 1656–81 or Marquis. That is, the Hope-1656-81-Marquis group of globulins is more specific than the globulins of the remaining common wheat, Hard Federation, and within the group the globulins of Hope are more specific than those of the other members of the group—all being estimated by titration against Mindum antigen.

(*c*) These antigens were obtained from hybrid wheats which represent numerous crosses. Evidence of their serological complexity shown in this figure and in the other four is in agreement with the genetic history of the hybrids.

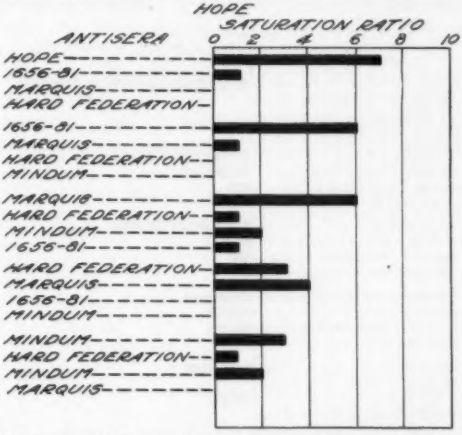

FIG. 3.—Results of absorption experiments using Hope as absorbing antigen. Antisera in order of application, using 1:80 dilution only: Hope (7); 1656-81 (1); Marquis (0); Hard Federation (0). 1656-81 (6); Marquis (1); Hard Federation (0); Mindum (0). Marquis (6); Hard Federation (1); Mindum (2); 1656-81 (1). Hard Federation (3); Marquis (4); 1656-81 (0); Mindum (0). Mindum (3); Hard Federation (1); Mindum (2); Marquis (0)

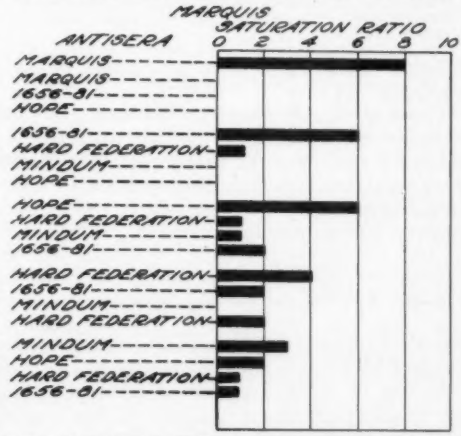

FIG. 4.—Results of absorption experiments using Marquis as absorbing antigen. Antisera in order of application, using 1:80 dilution only: Marquis (8); Marquis (0); 1656-81 (0); Hope (0). 1656-81 (6); Hard Federation (1); Mindum (0); Hope (0). Hope (6); Hard Federation (1); Mindum (1); 1656-81 (2). Hard Federation (4); 1656-81 (2); Mindum (0); Hard Federation (2). Mindum (3); Hope (2); Hard Federation (1); 1656-81 (1)

The objections to absorption tests such as these are: (1) The impossibility of detecting overlapping of precipitations due to characters possessed in common by two or more sera, and (2) exhaustion of the test solution (antigen) by dilution after the third or fourth addition. In none of the tests observed was there sufficient difference in the physical nature of the precipitates to validate objection to the use of bulk estimation as a quantitative measure of specificity.

DISCUSSION

The globulins characterizing wheat varieties may be ranked serologically in accordance with the percentage amount of precipitate formed, using the amount of precipitate from the homologous antigen-serum couple as a basis of comparison, as can be seen by examining the right-hand column of Tables 1 to 5, inclusive.

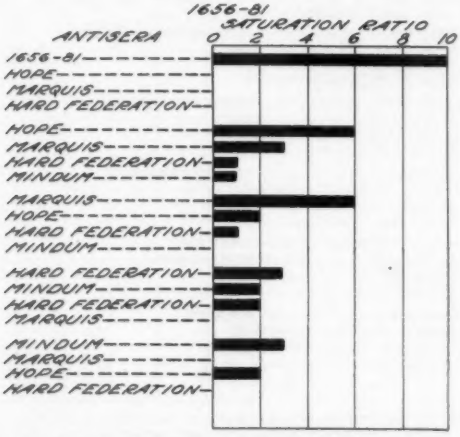

FIG. 5.—Results of absorption experiments using 1656-81 as absorbing antigen. Antisera in order of application, using 1:80 dilution only: 1656-81 (10); Hope (0); Marquis (0); Hard Federation (0). Hope (6); Marquis (3); Hard Federation (1); Mindum (1). Marquis (6); Hope (2); Hard Federation (1); Mindum (0). Hard Federation (3); Mindum (2); Hard Federation (2); Marquis (0). Mindum (3); Marquis (0), Hope (2); Hard Federation (0)

In Tables 1 and 2 the ranks are reciprocal. A close relationship is indicated between the durum wheat, Mindum, and the common wheat, Hope. But in the other tabulations the same serological relationship is not apparent. Marquis is a common parent of both Hope and 1656-81. This is indicated above in the close grouping of these three wheats in all five tabulations. Hard Federation, of different parentage, does not stand in such an immediate position serologically.

From the retabulation of Tables 1 to 5 (Table 8) it may be seen that common wheat antigens are approximately 0.29 as precipitable with Mindum antiserum as the homologous antigen is; and with common wheat antisera the Mindum antigen is 0.25+ as precipitable as any homologous couple of the same. Or, Mindum antigen is approximately 0.45 as precipitable (i. e., 25/55) as the common wheat group.

TABLE 8.—*Retabulation of Tables 1 to 5, inclusive*

Antiserum	Antigen					Average for common wheat precipitates	Average for Mindum precipitate
	Hope	1656-81	Marquis	Hard Federation	Mindum		
Mindum (durum)	39	37	27	14		29	
Hard Federation	30	49	64		19	47	19
Hope		63	54	25	27	47	27
Marquis	67	72		53	32	64	32
1656-81	83		63	37	23	60	23
General average of precipitability of heterologous common wheats						55	
General average of precipitability of Mindum by common wheat antisera							25+

The order of precipitability of the common wheat antigens with Mindum antiserum can be represented by a simple linear arrangement—Hope, 1656–81, Marquis, Hard Federation. As has been stated, the first three, mutually related to each other by hybridization, are closer serologically to Mindum than Hard Federation is, and in the linear arrangement Hope is nearest to Mindum.

In the present status of immunological science it is permissible to consider precipitability of an antigen with an antiserum as a measure of specificity of one for the other. Granting that as time goes on we may have to qualify this statement, it does not seem objectionable to rank these wheats in the order of the precipitability of their globulins in cross titration with the antiserum representing any one of the wheats. This ranking is synonymous with serological relationship.

As a result of this reasoning the serological relationship can be tabulated thus:

In order of serological relationship to—	Rank			
	1	2	3	4
1. Mindum	Hope	1656–81	Marquis	Hard Federation.
2. Hard Federation	Marquis	1656–81	Hope	Mindum.
3. Hope	1656–81	Marquis	Mindum	Hard Federation.
4. Marquis	1656–81	Hope	Hard Federation	Mindum.
5. 1656–81	Hope	Marquis	do	Do.

CONCLUSIONS

Globulinlike protein fractions can be extracted from wheats by means of appropriate mechanical and physical methods. These globulins are in a condition of sufficient purity to lend themselves to the serological differentiation of closely related hybrid wheats.

Varieties of wheat can be classified serologically, using any one variety as a standard. The varieties of wheat that possess the greatest number of genetic factors in common show the closest relationship serologically. In particular, among the five wheat hybrids studied there is a close serological relationship between Hope, 1656–81, and the common parent, Marquis; in cross titration with the various heterologous antisera, the antigens assume a definite linear relationship; in the linear relationship established by the heterologous antigens in titration with Mindum antiserum, the order is Hope, 1656–81, Marquis, and Hard Federation.

The serological relationship is correlated with or dependent upon the genetic composition of the variety. Since the genetic composition of the variety is responsible for its morphological as well as its physiological characteristics, the serological reactions may be used within limits to ascertain these characteristics. Saturation of homologous antigen with antiserum completely eliminates the specific characters by precipitation. In general, the titration of an antigen with an heterologous antiserum eliminates all the specific characters in the serum responsive to that antigen. The amount of precipitate in heterologous titration is greatest when the varieties used contain the greatest number of genetic characters in common.

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Absorption experiments indicate considerable serological overlapping. The precipitate represents the genetic character or characters common to the varieties concerned, such as rust resistance, hardiness, fruitfulness, etc. These varieties of wheat were originally selected from many that had been studied for resistance to stem rust. Hope is considered the most rust resistant of the five varieties dealt with in this paper. Mindum is next with somewhat less resistance. The apparent parallelism in serological relationship and rust resistance seems to offer data more hopeful for study than any that genetic or cytological research, such as chromosome determination, has so far produced.

SUMMARY

Evidence is presented to show that five well-known wheat varieties which were originally selected by plant breeders for such genetic characters as resistance to stem rust, yield, etc., may be ranked with reference to one another by means of the precipitin technic. To a certain extent the serological ranking is paralleled by the genetic characters for which the wheats were originally selected.

It is suggested that within limits, the use of the specific globulin fractions involved in this serological technic may aid the plant breeder by serving as serological indicators of special characters which are coincident with the inheritance of the specific protein make-up of the variety.

The extreme complexity of this protein structure within which the genetic complex of the variety is involved is indicated by a series of absorption tests.

The need of special care to avoid denaturing the specific protein fractions is emphasized.

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SOME EFFECTS OF THE WAXY GENE IN MAIZE ON FAT METABOLISM.[1]

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INTRODUCTION

Advance in our knowledge of the basic physiological activities of genes would seem to follow the establishment, first, of all, of the primary effects produced by them during development. Comparative physicochemical studies of mutant types which differ in one or more Mendelian factors have so far proved to be the most practical means of revealing the specific effects of separate elements. For this purpose genetic methods of analysis are not effective. By their means, however, the student may obtain the necessary material for an investigation of the physiological activities of genes during ontogeny.

Brink and Abegg (*3*)[3] have already emphasized the fact that waxy maize offers favorable material for a study of the gene as a dynamic unit, by reason of the fact that the waxy gene manifests its action in pollen. A study of the specific modifications produced by the waxy gene on the properties of the carbohydrate reserves of endosperm and pollen was undertaken to secure information about its physiological activities. The starchlike nature of the main carbohydrate reserve in waxy corn pollen and endosperm was indicated by the following properties: Its comparative nonsolubility in water and in 10 per cent alcohol, its separation into two fractions on centrifugation, and its behavior on hydrolysis with hydrochloric acid and amylase. Starches prepared from the waxy endosperm and the pollen of waxy plants gave identical color reactions with iodine.

The decrease in the dry weight of the seed reserves during germination in the dark was practically the same in the waxy as in the nonwaxy types. There was no marked difference in the rate of sprout development during the seedling stage. It was suggested that the kind of starch reserve synthesized in the endosperm is controlled by the kind or amount of amylase present; and that the substratum (starch) and the enzyme (amylase) bear such a relation to each other that hydrolysis proceeds at practically the same rate in nonwaxy as in waxy seedlings. The hypothesis was advanced that the waxy gene primarily governs changes in the metabolism of the starch reserves of corn endosperm and pollen through an initial effect on the type or amount of amylase formed.

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[1] Received for publication Oct. 4, 1928; issued February, 1929. This paper was submitted in thesis form to the graduate school of the University of Wisconsin in partial fulfillment of the requirements of the degree of doctor of philosophy, March, 1918. Paper No. 94 from the department of genetics, Agricultural Experiment Station, University of Wisconsin. Published with the approval of the director of the station.

[2] Grateful acknowledgement is made to Dr. R. A. Brink, under whose supervision this investigation was made, for his generous and helpful criticism. The writer is also indebted to Dr. L. J. Cole for aid in this investigation.

[3] Reference is made by number (italic) to "Literature cited", p. 193.

The question arose whether the waxy gene is limited in its sphere of action to changes in carbohydrate metabolism and it seemed to the writer that the waxy gene might occasion attendant changes in fat metabolism as well. Comparative chemical analyses of the ether-soluble substances of nonwaxy and waxy types of maize grain and pollen were therefore undertaken in 1925. The results of these and subsequent analyses, which are presented herewith, show that the waxy gene produces marked effects on the fat metabolism. These effects are evident, however, only in the endosperm tissue. What the relationship is between the effects of this gene on both carbohydrate and fat metabolism remains unknown.

EXPERIMENTAL METHODS

SOURCE OF SEED AND POLLEN

Nonwaxy and waxy seed classes resulting from selfs of *Wxwx* plants (seeds from Ohio ear No. 1) were planted in 1924 and the *WxWx* individuals were separated from the *Wxwx* sibs by means of the pollen iodine test. This afforded a means of securing homozygous lines of nonwaxy and waxy seed derived from sister progenies. The resulting seed from second-generation selfs and from intraline crosses of these related strains were used for the chemical analyses in experiment 1. The stock of nonwaxy and waxy seed used in experiment 2 was derived from five sister plants which were the seed parents in the back cross *wxwx* ♀ × *Wxwx* ♂. Stock 2 is not closely related to stock 1. The material used in experiment 3 came from reciprocal back crosses, and is not related to stock 2. The results of fat analyses of these stocks show differences of such magnitude between the nonwaxy and waxy types that diversity in origin of stocks is obviously not a conditioning factor. Within each stock the two seed classes used came from closely related progenies. In cases where the two seed classes came from the same ear, development occurred in a uniform environment. Corn pollen for chemical analyses was collected from sets of nonwaxy and waxy plants grown from the corresponding seed classes on selfed ears.

CHEMICAL METHODS

After collection the pollen was dried for 24 hours at a temperature of 38° to 40° C. Dessication was completed in vacuo. Previous to fat extraction the pollen was passed through a 200-mesh sieve. The ether used in the oil and fat extractions of the ground maize grain and of pollen was practically free from alcohol, water, and acid. The Caldwell extractor was employed for all but a few large samples. The extraction was continued for 22 hours, at a temperature of 52° C. The solvent was evaporated from the residue at a low temperature. The residue was finally placed over c. p. concentrated sulphuric acid and dried to constant weight in vacuo. The percentage of extract was based on the dry weight of the maize grain and pollen. Moisture determinations of corn meal are in no sense absolute. Therefore, a 12-hour period of drying in an electric oven at 100° C. was arbitrarily chosen. The dry weight of pollen was estimated after reduction to constant weight in vacuo. As a rule all moisture determinations were made in duplicate.

Standard methods described by Leathes and Raper (*8*) and by Lewkowitsch (*9, v. 1*) were adopted with some necessary modifications for determining the properties of the crude oil and fat. Acid values were obtained by taking up the dried extract in warm, recently neutralized, 95 per cent alcohol. The normality of the alkali solution used in titration varied from N/100 to N/10 potassium hydroxide, depending on the amount of free fatty acids present in the extracts. Where essential, blanks were run. Saponification values were determined by refluxing the samples for 30 minutes with an appropriate amount of alcoholic potash of known standard strength, usually near N/2. Two blank determinations were made in all cases. Back titrations with N/2 HCl were made when necessary.

EXPERIMENTAL DATA

CRUDE-OIL DETERMINATIONS OF ETHER EXTRACTS OF NONWAXY AND WAXY TYPES OF MAIZE GRAIN AND POLLEN

The crude-oil percentages recorded in Table 1 are based on the dry weight of the various samples. In all cases, except the three embryo lots in experiment 3, the figures cited in Table 1 are averages of duplicate determinations showing good agreement. In each experiment paired nonwaxy and waxy determinations labeled a, b, and c represent analyses of samples from different pairs of ears. The three pairs within the same stock are closely related to each other.

TABLE 1.—*Percentage of crude oil in nonwaxy and waxy whole maize grain, endosperm, and embryo*

Experiment No.	Type of maize	Percentage of crude oil in—								
		Whole-grain samples			Endosperm samples			Embryo samples		
		a	b	c	a	b	c	a	b	c
1	Nonwaxy	7.18	5.97	7.08						
	Waxy	8.68	6.60	7.94						
3	Nonwaxy	5.67			0.98	1.04	1.18	33.77	33.4	34.8
	Waxy	6.16			1.46	1.38	1.71	36.76	32.2	34.0
Average	Nonwaxy	6.50			1.05			34.0		
	Waxy	7.30			1.50			34.3		

The yield of crude oil from the waxy grain is on an average 12.3 per cent greater than that from the nonwaxy. The odds are about 5,000 : 1 that the mean difference in yield between these types is significant (*9 contrasting pairs of determinations—Student's method (1)*). Variability in crude oil difference is great, but the waxy exceeds the nonwaxy in all cases.

It is evident from the figures recorded in Table 1 that no consistent and significant difference in crude-oil content exists between the embryo of the waxy and nonwaxy types. The observed difference in crude-oil yield between the whole-grain waxy and nonwaxy samples is not referable to their embryo tissues but to their endosperm tissues. The absence of any striking difference in the percentage of nonwaxy and waxy embryo crude oil seems to have peculiar significance when

this result is considered in relation to the fact that both the nonwaxy and waxy embryo tissues reveal only blue starch granules. The waxy gene evidently produces no demonstrable effect on carbohydrate and fat metabolism in the embryo tissue of the grain.

The endosperm fractions [4] used for ether extractions were quite free from embryo tissue. The latter is very rich in oil and small amounts of it in admixture with endosperm tissue would introduce a large error. The percentage increase in yield of ether extract from waxy endosperm tissue over that of the nonwaxy is nearly 43. Six pairs of comparable samples were analyzed. The odds are very large that the mean difference is significant. No other analysis of this nature has been made on waxy endosperm tissue, so far as we are able to determine. The nonwaxy ether extract analyses are in agreement with those cited by Atwater (*2*), and Lindstrom and Gerhardt (*10*). The results up to this point lead to the conclusion that the difference in the character of the ether extracts is caused by differences in the fatty substances of the endosperm tissues. The activities of the waxy gene extend to some phases of the endosperm fat metabolism.

The fact that the waxy gene modifies the pollen starch reserves and the waxy endosperm reserves in a similar manner has been determined by Demerec (*5*), and Brink and MacGillivray (*4*). This suggested the possibility that there might be a parallel between pollen and endosperm in regard to differences between nonwaxy and waxy types in yield of either extract. However, the evidence on this point is negative. Direct ether extraction of related nonwaxy and waxy pollen produced an average yield of 0.64 per cent in the case of the former and 0.67 per cent in that of the latter. This is an increase in the waxy class of only 5 per cent. There was no consistent difference in yield between these types of pollen. Further extractions will be necessary to test the hypothesis that the activities of the waxy gene modify fat metabolism in pollen.

COMPARATIVE ACID, SAPONIFICATION, AND ESTER VALUES OF ETHER EXTRACTS OF NONWAXY AND WAXY TYPES OF MAIZE GRAIN AND POLLEN

TABLE 2.—*Acid values of the ether soluble crude fats from whole maize grain, endosperm, and embryo samples*

Experiment No.	Type of maize	Acid numbers of—									
		Whole-grain samples				Endosperm samples			Embryo samples		
		a	b	c Yellow	c' White	a	b	c	a	b	c
----	Nonwaxy	14.0	12.2	9.3	11.3	----	----	----	----	----	----
		14.1	12.3	10.0	----	----	----	----	----	----	----
	Waxy	27.6	43.0	29.6	31.0	----	----	----	----	----	----
		28.0	39.1	29.5	32.1	----	----	----	----	----	----
----	Nonwaxy	17.8	----	----	----	37.6	48.0	49.3	----	----	----
		18.0	----	----	----	40.4	47.3	48.6	2.8	4.8	5.1
	Waxy	38.4	----	----	----	76.0	80.9	87.3	----	----	----
		----	----	----	----	79.5	83.0	86.1	2.6	5.2	4.7
Average	Nonwaxy	13.0				45.0			4.2		
	Waxy	34.0				82.0			4.1		

[4] In endosperm fractions a small amount of pericarp tissue is present, which does not affect the difference in endosperm crude fat yield.

The figures in Table 2 indicate that the average acid number (measure of free fatty acids) for the nonwaxy whole grain crude oil is 13 and that for the waxy 34. It therefore took 160 per cent more caustic potash to neutralize the acid substances in the waxy than in the nonwaxy crude oil. Blanks were run with these extractions, but the weights of these check residues and the amount of potassium hydroxide solution required to neutralize them were negligible. The difference between the nonwaxy and waxy crude oil acid numbers is an extremely variable one, but in all cases the waxy number exceeds that of the nonwaxy type. The data in Table 2 on whole-grain acid numbers indicate that in both the nonwaxy and waxy classes the white grain (*y*) is slightly higher in acid number than the yellow type (*Y*). The indications are that the effect of the waxy gene on acid value is independent of the action of the recessive *y* gene on fat metabolism.

Whereas the acid values of the crude oils from the nonwaxy and waxy embryo tissues are practically alike, the endosperm values of these types differ strikingly. An examination of the literature has failed to reveal the existence of acid-value figures on crude oil extracted from dry endosperm tissue (i. e., no soaking of seed to expedite separation of endosperm and embryo). It is probable that the higher acid value of the waxy endosperm crude oil as contrasted with that of the nonwaxy denotes a greater content of free fatty acids, per unit quantity, in the former. This difference is evidently restricted to the endosperm ether extracts. This agrees with the conclusion reached regarding differences in the yield of crude oil.

The difference in acid value between the nonwaxy and waxy endosperm crude fats is not a constant one. In stock 2 the nonwaxy (*Wxwxwx*) and the waxy (*wxwxwx*) endosperm types gave acid values of 76 and 117, respectively. Corresponding values of the samples (*Wx Wxwx* and *wxwxwx*) in stock 3 were 45 and 82. The two stocks may differ in subsidiary factors which modify the degree of the acid-value difference. Possibly the greater increase of the waxy type over the nonwaxy in experiment 3[5] as contrasted with that in experiment 2 may have some connection with a dosage difference of the nonwaxy—waxy factor pair existing between these stocks. The causal factors associated with marked variability in acid values remain unknown.

The acid values of crude fats from related nonwaxy and waxy pollen samples were 42 and 43, respectively. Difficulties in technic were encountered and poor agreement was found in duplicates. If there is any difference at all between the ether-soluble free fatty acids of these pollen types, it is a small one. The result obtained is in decided contrast with that of Demerec (*5*) and Brink and MacGillivray (*4*), who have shown that the activities of the waxy gene are manifested on carbohydrate metabolism in the gametophyte generation. A statistically significant difference between the nonwaxy and waxy types in ether extract yield and in acid value seems to be characteristic of the crude fats from endosperm tissue only.

A sufficient quantity of isolated dry endosperm tissue was on hand from stocks 2 and 3 for single determinations, in each case, of the saponification values of nonwaxy and waxy crude fats. This value is a measure of the total fatty acids in unit quantity of a fat or oil, and

[5] Stock and experiment numbers are identical.

is therefore inclusive of both combined and free fatty acids. Data in Table 3 show that the average saponification value of the crude fat from the waxy endosperm is 200, taken to the nearest whole number, while that from the nonwaxy endosperm is 177. The presence of unsaponifiable constituents in ether extracts effects a lowering of the true saponification value of the fat and oil present. The removal of the former substances was not practicable, for at best endosperm tissue yields only small amounts of crude fat. Either the crude fat from the nonwaxy endosperm is higher in unsaponifiable substances than that from the waxy endosperm or else the fatty acids in the nonwaxy crude fat have a higher mean molecular weight than those of the waxy. The isolation of the fatty acids from the crude fats of the nonwaxy and waxy endosperm is necessary to settle this question.

TABLE 3.—*Saponification and ester values of crude fat from nonwaxy and waxy endosperm*

Experiment No.	Type of maize	Saponification value	Acid value	Ester value [a]
2	Nonwaxy	176	76	100
	Waxy	205	117	88
3	Nonwaxy	178	45	133
	Waxy	195	82	113
Average	Nonwaxy	177	60	117
	Waxy	200	100	100

[a] Difference between saponification and acid values.

The difference between the saponification and acid values is called the ester value and is a measure of the combined fatty acids in unit quantity of a sample of fat. The ester value of the waxy type fat (Table 3) is appreciably lower than that of the nonwaxy type. This may indicate that crude fat from the waxy endosperm contains less neutral fat and more free fatty acids than that from nonwaxy endosperm tissue. It may be assumed that in the fat metabolism of the waxy endosperm tissue a lipase is active which does not possess the capacity to form neutral fat to the same extent as nonwaxy lipase. It may prove, however, to be a question of the relative amounts of some lipase inhibiting substances controlling the fat metabolism in nonwaxy and waxy types of maize.

INCREASE OF ACID VALUES OF CRUDE OILS FROM GROUND MAIZE GRAIN DUE TO STORAGE

In repeating ether extractions and acid-value determinations upon various samples of endosperm meal which had been stored for some time in well-corked glass bottles, it was found that in all cases the acid values of the crude fat from such stored samples were strikingly higher than those obtained on fat from earlier ether extracts. Moreover, this rise in acid value was specific for each type. Ether extractions were then made of ground samples of nonwaxy and waxy whole grain which had been stored for approximately four months. These samples from stock 1 had previously served as sources of crude oil for the determinations recorded in Table 2. In Table 4 these values are compared with those of the crude oils extracted four months later.

TABLE 4.—*Rise of acid values as a consequence of storage of ground nonwaxy and and waxy maize grain*

Type of maize	Acid value determination in—		Percentage increase of b over a
	November, 1926 (a)	March, 1927 (b)	
Nonwaxy	10	30	200
Waxy	30	106	253
Nonwaxy	12	50	316
Waxy	40	124	210

The acid values of the nonwaxy type of grain have increased to a greater extent than those of the waxy type, during an e qual period of time in storage. The difference in acid value between the two types has been reduced. Wilk, without considering temperature (*13*), finds that the acidity of a feed meal is a function of age and that the acidity increases gradually with the catalytic effect of fat-splitting enzymes. According to this author various grain meals have specific acidities, presumably controlled by the activities of corresponding specific lipases. It is probable that the difference in the percentage increase between the acid values of the nonwaxy and waxy types indicates a difference in the fat-splitting powers of their respective endosperm lipases. The nonwaxy and waxy genes may exert differential influences on reactions related to the hydrolytic powers of lipase.

DISCUSSION

The purpose of this investigation is to extend our knowledge of the primary effects of the waxy gene on the reserves in maize grain and in pollen. The determination of these specific effects seems to be a necessary first step toward a complete understanding of the important physiological properties of the waxy gene. In a previous investigation by Brink and Abegg (*3*), attention was centered upon the nature of the carbohydrates stored in the endosperm and pollen of the waxy type as compared with that of the corresponding reserves in the nonwaxy type. In 1925 such comparative analyses were extended by the writer to the ether soluble substances, principally those from the endosperm tissues.

Ether extraction of comparable lots of nonwaxy and waxy maize grain have shown that the average yield of the former is 6.5 per cent of crude oil and of the latter 7.3 per cent. The difference is statistically significant. This difference applies only to ether extracts from the endosperm tissues. The nonwaxy endosperm tissue yielded an average of 1.05 per cent of crude fat, and the waxy, 1.50 per cent. The average yield of crude oil from waxy embryos was 34.3 per cent and that from the nonwaxy, 34 per cent. The latter difference is not significant. The increased yield in endosperm ether extract from the waxy type probably consists largely of free fatty acids. The high saponification values of the crude fats from nonwaxy and waxy endosperm indicate that fatty acids, combined and free, are the common components of such ether extracts. In addition, the higher acid value of crude fat from the waxy endosperm suggests that the

increased yield of crude fat is in this case largely composed of free fatty acids.

The average acid value (measure of free fatty acids) of the ether soluble oil from nonwaxy maize grain is 13 and that of comparable waxy oil 34. This difference is localized in the crude fats from endosperm tissue. In one stock, a unit quantity, of the crude fat from the waxy endosperm required 82 milligrams of potassium hydroxide to neutralize its free fatty acids, while the same amount of nonwaxy fat required only 45 milligrams. In another unrelated lot of comparable samples corresponding acid values of 117 and 76 were obtained. That these differences are restricted to the endosperm crude fats is clearly indicated by the fact that in the first-named stock the average acid value for nonwaxy embryo crude oil was 4.2 and that for the waxy 4.1.

The average saponification number (measure of total fatty acids in unit quantity of fat) in the above-mentioned two stocks was found to be 177 for the nonwaxy endosperm crude fat and 200 for the waxy. The corresponding ester values which measure combined fatty acids proved to be 117 and 100, respectively. The waxy endosperm crude fat, per unit quantity, is lower in neutral fat and higher in free fatty acids than an equivalent amount of nonwaxy crude fat.

That the differences in the amount and in the acid values of extracted crude fats should be localized in the endosperm tissues accords with the previously established knowledge that only the endosperm starches of these types of maize grain differ in their reaction to iodine. The waxy endosperm starch acquires a red-violet color when treated with potassium iodide which is clearly differentiated from the blue color developed by nonwaxy starch. On the other hand, the starch granules in both types of embryos react alike with iodine, both giving a blue color. The occurrence of differential activities of the nonwaxy and waxy genes in both carbohydrate and fat metabolism is demonstrated by data on nonwaxy and waxy endosperm starch and crude-fat differences.

These data invite speculation on the manner in which the waxy gene operates to modify the yield and properties of ether soluble crude fat from the endosperm. Such speculations on differences in yield are cut short, for preliminary analyses have shown that ether extraction yields approximately only from 43 to 44 per cent of the total crude fat content of maize grain, irrespective of type. This statement is based on results from alcoholic extractions of nonwaxy and waxy grain. Until accurate data are available, based on total crude fat content it may be stated that a differential yield of ether-soluble nonwaxy and waxy endosperm crude fat is associated with the *Wx–wx* factor difference.

Results established on differences in properties of nonwaxy and waxy endosperm crude fats lead to the belief that the waxy gene may modify, directly or indirectly, the activity of maize endosperm lipase, one of the functions of which is the condensation of fatty acids and glycerol to form neutral fat. The lower ester value (measure of combined fatty acids) and the higher acid value of waxy endosperm crude fat as compared with the nonwaxy reserve may be an index to differences between these types in either the rate of the synthetic activity of their lipases or in their content of an antienzyme. Data which indirectly confirm this hypothesis are available from acid values determined on alcoholic extractions of ground nonwaxy and waxy whole

grain. The difference between these types in acid value, using absolute alcohol as a solvent, is still evident though somewhat reduced in degree. The differential influence of the *Wx–wx* factor pair may also include the hydrolytic activities of nonwaxy and waxy lipase. The acid values of nonwaxy and waxy crude fats extracted from maize grain meals were determined immediately after grinding and also after a period of storage. The crude fat from nonwaxy stored grain meal showed a greater increase in acid value than that from stored waxy grain meal. This may indicate that fat hydrolysis in these types of maize proceeds at different rates. Unpublished comparative data secured by the writer on acid values of crude fats extracted from waxy, sugary, and sugary-waxy endosperm meals, before and after storage at room temperature for an equal period of time, show that a marked rise in acid value occurs in each case after storage. The degree of increase was found to be specific for each type of endosperm meal. It seems probable, therefore, that the phenomenon of differential increase in acid value, contrasting crude fats from stored nonwaxy grain meals, is connected with a difference in their endosperm fat metabolism.

There is no evidence from the results in this investigation that the waxy gene possesses properties which place it in the category of an autocatalyst, nor is it reasonable to assume that this gene is an enzyme. In this connection it is interesting to note that Goldschmidt (*6*, *7*) has formulated a sex-factor enzyme hypothesis from results on genetic studies of intersexuality in *Lymantria dispar*. This hypothesis was extended to include all Mendelian factors and assumes that genes are enzymes or similar substances. The author has attempted to place sex differentiation in *L. dispar* on a definite factor basis. There is a division of opinion on the existence of specific heritable factors for maleness and femaleness. Moreover, no actual physicochemical data have been offered by Goldschmidt (loc. cit.) in support of the idea that genes are enzymes. Morgan (*11*) advances the provisional conclusion that while we may not be warranted in speaking of the genes as enzymes, they may be protein bodies one function of which is to produce enzymes which take part in catalytic reactions. Schmalfusz and Werner (*12*) have advanced a somewhat similar working hypothesis. Their chemical interpretation of the production of Mendelian characters seems plausible enough, but the characters given are hypothetical.

The comparative analyses of nonwaxy and waxy maize pollen, as far as yield and acid values of their ether soluble crude fats are concerned, have failed to reveal any striking difference between these types. The average yield and acid value of the ether-soluble crude fat from the waxy type pollen was slightly higher than that from the nonwaxy pollen, but the difference does not appear to be significant. This result is in marked contrast with the situation in the nonwaxy and waxy endosperm tissue. These particular pollen analyses emphasize the conviction that the primary effect of the waxy gene is probably one regulating carbohydrate metabolism. The investigations of Demerec (*5*) and Brink and MacGillivray (*4*) have demonstrated that the waxy gene manifests its activities in pollen and in endosperm tissue in a similar manner. Waxy pollen and endosperm starches both give an identical red-violet color with

iodine. Further, Brink and Abegg (*3*) have shown that in other properties these starches may be considered identical. In view of the available evidence it is probable that the marked effects of the waxy gene on the fat metabolism of the endosperm are accessory and are to a large extent peculiarly restricted to that tissue.

There is a need for the extension of physicochemical analyses to the protein and mineral metabolism of related nonwaxy and waxy types of maize endosperm and pollen. More complete information on the manifold effects of the waxy gene may go far toward a description of the primary physiological properties of this heritable element.

SUMMARY

Ether extraction of comparable samples of nonwaxy and waxy maize grain have shown that the former yields, on the average, 6.5 per cent of crude oil and the latter 7.3 per cent. The difference is statistically significant.

The yield of ether-soluble crude fat from nonwaxy endosperm tissue proved to be 1.05 per cent and that from closely related waxy endosperm tissue 1.5 per cent. The nonwaxy embryo tissue yielded 34 per cent of crude oil and the waxy 34.3 per cent. The difference in crude oil yield between these embryo types does not appear to be significant.

The average acid value (measure of free fatty acid) of the ether soluble crude oil from nonwaxy maize grain was found to be 13 and that of the waxy 34. This difference was reduced where absolute alcohol was used as solvent, although the waxy sample still measurably exceeded the nonwaxy in acid value.

The average acid values of the ether-soluble crude fats from two different stocks of nonwaxy endosperm tissue were found to be 76 and 45, and those of the waxy 117 and 82, respectively. In one of these stocks the average acid value of the nonwaxy embryo crude oil was 4.21 and that of the waxy 4.16.

The average saponification number of crude fat from the nonwaxy endosperm was found to be 177 and that from the waxy 200. The corresponding ester values were 117 and 100, respectively.

The characteristic differences between the nonwaxy and waxy endosperm tissues in yield of crude fat and in acid value are not evident between nonwaxy and waxy maize pollen.

It is concluded that a marked difference between the nonwaxy and waxy types in yield of crude fat and in acid value is restricted to the endosperm tissue.

The evidence adduced indicates that the waxy gene in maize produces marked effects upon the fat metabolism of endosperm tissue. Apparently this gene is not limited in its sphere of action to changes in the carbohydrate metabolism alone. Since the effects of the waxy gene on carbohydrate metabolism are evident in both endosperm and pollen and this does not prove to be the case with its effect on fat metabolism, the former may be considered as primary and the latter as secondary.

The following working hypothesis is advanced. The activities of the waxy gene seem to be connected with the synthetic and hydrolytic powers of maize endosperm lipase. It appears that the waxy type lipase does not effect a condensation of fatty acids with glycerol to

the same degree as does the nonwaxy. Hence the waxy endosperm contains a crude fat whose acid value is higher than that from the nonwaxy type. Moreover, the fact that the acid value of the crude fat from stored waxy type maize grain meal, although showing a great increase does not equal that made by the fat from nonwaxy meal following storage, suggests the possibility that the waxy lipase is inferior to that of the nonwaxy in hydrolytic power.

An extension of physicochemical analyses to the protein and mineral metabolism of related nonwaxy and waxy types of maize endosperm and pollen is suggested. This would seem to be a necessary preliminary step toward a complete understanding of the primary physiological activities of the waxy gene during development.

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